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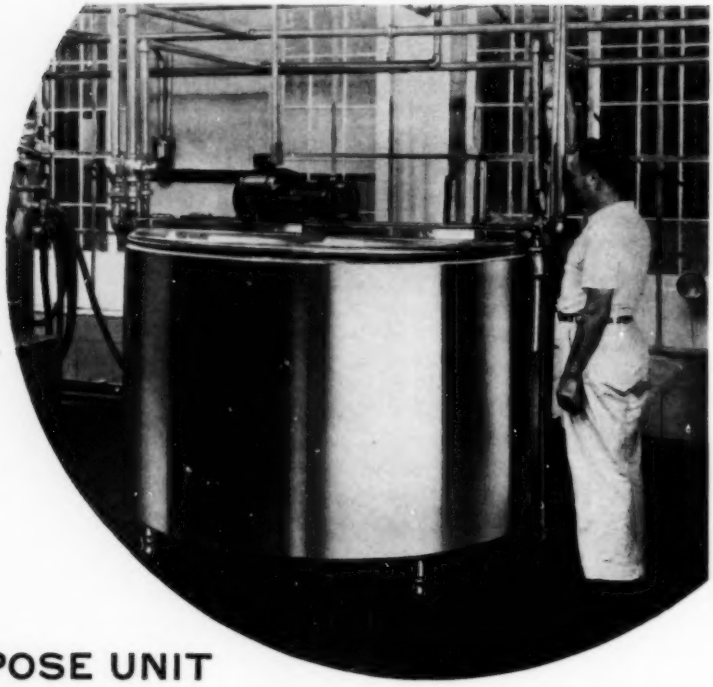

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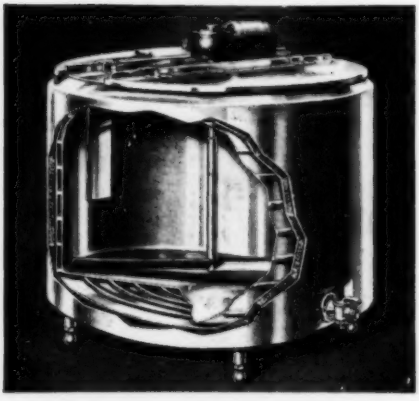

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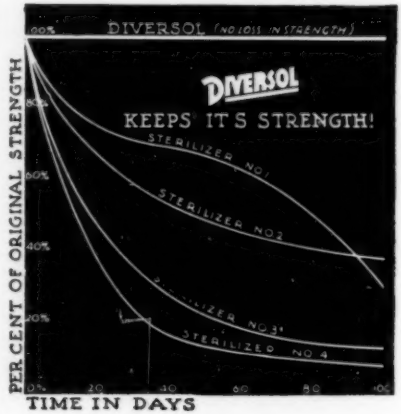

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JOURNAL OF DAIRY SCIENCE

VOLUME XIX DECEMBER, 1936 NUMBER 12

CLEANING DAIRY EQUIPMENT WITH TRISODIUM PHOSPHATE

L. A. ROGERS AND FRED R. EVANS

Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

When the hand separator displaced to a large extent the factory separation of milk, the quality of the cream for buttermaking was seriously impaired. This was due partly to the less frequent deliveries to the creamery and partly to the failure to properly clean and sterilize the separators on the farm. In some cases improper cleaning is due to negligence, but frequently it may be ascribed to the inherent difficulties in making bacteriologically clean an apparatus with so much surface and so many corners to hold moisture.

Milking machines are also difficult to keep properly cleaned and free from bacteria, and it is well known that under some conditions they may cause serious contamination of the milk.

Dairy utensils even when properly cleaned of visible dirt may harbor large numbers of bacteria which, if the temperature is favorable, may multiply enormously in the film of water left on the surfaces. Not only is it difficult to keep a separator properly cleaned under the best conditions, but, due to the methods followed by many creameries in buying cream, careless habits have developed and the separator may be washed only once each day, and in some cases less frequently.

SEPARATORS

The effect of improper care of the separator is illustrated by Table 1. In this case the hand separator was washed and steamed after the morning separation, but in the evening was allowed to stand without washing and used in the morning to separate a fresh batch of milk. The bacteria count of the milk was determined in the milk before separation and in the mixed milk and cream after separation. For this purpose milk powder-tomato juice agar incubated at 30° C. was used.

While the evening separation with the properly cleaned separator caused little or no increase in bacteria in the milk, the morning separation, after the separator had stood over night without cleaning, resulted in a marked increase.

Received for publication July 2, 1936.

TABLE 1

Increase in bacteria counts due to separation. Separator used twice daily, washed only after morning separation

DATE	MORNING		EVENING	
	Before separation	After separation	Before separation	After separation
Oct. 8	1,600	2,900	5,000	6,100
9	8,800	125,000	130,000	120,000
10	6,300	390,000		
11	6,700	37,000	34,000	72,000
14	18,000	31,000	39,000	36,000
Average	8,280	117,180	49,500	58,520

This effect will vary with the temperature of the room, the time the separator is allowed to stand uncleaned, and the original condition of the milk.

Any system designed to improve the condition of farm separators and milking machines must be inexpensive and should lessen rather than increase the labor. In attempting to devise a new method of cleaning this type of equipment to meet these requirements, we have taken advantage of the well-known bactericidal action of alkaline washing powders when used in solutions having a pH of approximately 11.0 or higher. Trisodium phosphate was chosen because, while it is an excellent detergent, its corrosive action is less than that of some of the other alkalies.

A five per cent solution of trisodium phosphate has a pH of 10.9. Increasing the strength of the solution to 10 per cent produces a change in the pH of only 0.3.

The corrosive action of trisodium phosphate may be reduced by the presence of small amounts of sodium chromate,[1] sodium perborate,[2] alkaline sodium metasilicate,[3] and other similar substances.

The protective action of these three chemicals against the corrosive action of a five per cent solution of trisodium phosphate on tin plate is illustrated in Table 2. This test was made by cutting ordinary tin plate in strips 7/8 x 1–1/2″ and submerging them in a five per cent solution of trisodium phosphate with various combinations of sodium chromate, sodium perborate, and sodium metasilicate, as shown in the Table. To be strictly comparable the comparisons should be made on the basis of surface exposed but since the strips of metal were of almost identical size the relative loss of weight is sufficiently accurate for this purpose.

[1] The use of combinations of sodium chromate, sodium silicate, and trisodium phosphate is protected by United States patents.

[2] Hunziker, O. F. The Butter Industry. Second edition. 1927, p. 58.

[3] Vail, James G. Some less familiar applications of soluble silicates. Ind. and Eng. Chem., 22: 1930; 972–974.

TABLE 2

Retardation of the corrosive action of trisodium phosphate on tin plate by sodium chromate, sodium perborate, and sodium metasilicate

TRIAL NO.	TRISODIUM PHOSPHATE SOLUTION CONTAINING			LOSS OF WEIGHT		
	Sodium chromate	Sodium perborate	Sodium metasilicate	7 days	14 days	21 days
	%	%	%	%	%	%
1	—			.038 (2)	.070	.147
2	0.5			.008 (2)	.018	.021
3	3.0			.000 (1)	.000	.000
4	5.0			.000 (1)	.000	.000
5		1		.018 (2)	.047	.086
6		5		.036 (2)	.057	.097
7		10		.042 (2)	.068	.099
8		20		.033 (2)	.058	.090
9			1	.041 (2)	.068	.111
10			5	.028 (2)	.044	.072
11			10	.026 (2)	.040	.080
12			20	.028 (2)	.048	.077
13	0.5	3		.008 (3)	.018	.024
14	0.5	5		.005 (3)	.009	.013
15	0.5		3	.011 (3)	.018	.023
16	0.5		5	.009 (3)	.015	.020

(1) Tin bright.
(2) Tin mottled like galvanized iron.
(3) Black patches usually beginning at the edge; remainder of tin bright.

In Table 2, Trial 1 shows the extent of corrosion by the five per cent solution of trisodium phosphate alone. When one-half of one per cent of sodium chromate was added this effect was materially reduced but not entirely eliminated; but with three and five per cent addition the loss in weight became less than one milligram in 28 days from metal weighing about 2.5 grams. Part of this loss could be accounted for by the cleansing action of the solution, but in the three per cent solution there was eventually a slight but distinct action made evident by dark spots on the tin.

Sodium perborate, sodium metasilicate, and combinations of the two had some protective action, but in none of the concentrations tried was it sufficient. It was observed that when the perborate or metasilicate was used with trisodium phosphate the corrosive action of the phosphate was evident in a mottled appearance of the tin, giving it the appearance of galvanized iron, while if one of the two was used in combination with chromate the tin remained bright except where dark patches developed.

In our experimental work a solution was used containing trisodium phosphate and sodium chromate in the following proportions:

Trisodium phosphate	5.000 grams
Sodium chromate[4]	0.150 "
Water	100.000 "

In view of the slightly corrosive action of the three per cent solution, it would probably be advisable in practice to use a solution containing five pounds of sodium chromate in each 100 pounds of trisodium phosphate.

Rubber tubing submerged in the trisodium phosphate-chromate solution for months showed no indication of any deterioration.

The labor involved in using a cleaning solution in the usual way was reduced by omitting any washing, scrubbing, or steaming of the separator parts, and substituting a rinsing of the parts in tap water, submerging in the trisodium phosphate-chromate solution until the next separation, rinsing in water to remove the solution, and assembling the parts. With this treatment any slime or sediment adhering to the bowl is loosened and remains in the solution, leaving the metal clean in appearance and nearly free from bacteria.

Soldered joints are darkened by this solution, and if any part is not completely submerged a dark line will be formed at the surface of the liquid,

TABLE 3

Increase in bacteria counts due to separation. Separator parts kept in bisodium phosphate-chromate solution between separations

DATE	MORNING		EVENING	
	Before separation	After separation	Before separation	After separation
Oct. 15	18,000	26,000	26,000	29,000
16	7,100	14,000		
17	15,000	19,000		
18	2,300	2,700		
21	7,000	6,500	4,100	3,600
22	7,900	6,500		
23	6,700	5,300	13,000	12,000
24	3,700	4,200	3,300	3,100
25	28,000	19,000		
28	5,500	11,000	20,000	21,000
30	3,100	2,900	13,000	8,000
Nov. 1	4,600	4,200		
4	9,000	6,500	2,300	2,500
6	14,000	11,000	13,000	11,000
7	59,000	58,000	63,000	65,000
12	10,000	9,800	9,600	8,400
Average	12,556	12,912	16,730	16,360

[4] It has been stated that some people are sensitive to sodium chromate and that a skin eruption results from its use. We have not observed this in our experience and, since it is not necessary to submerge the hands in the solution, trouble from this source would not be expected.

evidently through electrolytic action, but otherwise the appearance of the metal is unchanged. It may be advisable to occasionally scrub the separator parts with a stiff brush using a good washing powder to restore the original bright appearance.

The effect of this treatment on the bacteria count of the milk is shown in Table 3.

All factors considered, these counts show that a separator can be maintained in a satisfactory bacteriological condition by keeping the parts immersed in a solution of trisodium phosphate, while time and labor required in cleaning the separator are actually reduced.

In this experiment the separator was used twice daily and after each separation was taken down, rinsed in tap water, and submerged in the solution. At the next separation the parts were removed from the solution, rinsed in ap water, and assembled. This machine received no other treatment for the duration of the experiment.

It will be noted that in nearly every case the variation in bacteria count was well within the limits of experimental error.

MILKING MACHINES

In testing the efficiency of this system on a milking machine, a somewhat different technique was required. After each milking, the machine was rinsed in clean water and all parts, including the tubes and teat cups, but excluding the pail, were submerged in the trisodium phosphate-chromate solution. Immediately before the next milking these parts were rinsed with boiled water to remove the cleaning solution, and 2500 cc. of sterile water drawn through the machine. Bacteria counts were made on this water. In obtaining the data shown in Table 4 the machine received no other

TABLE 4

Bacteria count of rinse water from milking machine

DATE	TRISODIUM PHOSPHATE TREATMENT	CHLORINE TREATMENT	DATE	TRISODIUM PHOSPHATE TREATMENT	CHLORINE TREATMENT
July 24	1,300	14,000	Sept. 20	2,100	2,400
26	410	1,900	25	38,000	30
30	150	7,500	27	430	2,800
Aug. 20	240	5,300	Oct. 1	180	820
23	70,000	2,400	4	110	1,300
28	14,000	3,100	8	20	16,000
30	42,000	2,100	11	420	17,000
Sept. 4	4,100	7,300	15	230	14,000
6	370	1,500	18	360	30,000
10	38,000	90	22	10,000	25,000
13	1,400	1,700	25	50	17,000
17	3,000	2,000	29	80	8,300
			Nov. 1	40	9,400

treatment in the three months' period. In actual practice it would not be necessary to use boiled water if a water of good quality is available.

In the period from July 24 through August 30, the solution was not changed. It was renewed on August 30, and again on September 25. After this time it was renewed every seventh day. Examination of the solution indicated that the poor results obtained in August and September were due, not to an exhaustion of the alkali by long use, but to a gradual accumulation of bacterial spores. In October when the solution was renewed every week much lower counts were obtained than with the control chlorine treatment.

The results in the column headed "chlorine treatment" were obtained on a duplicate machine given the standard treatment used on the farm at that time.

The trisodium phosphate treatment had no apparent effect on the parts of the machine except that the handle on the top was darkened and slightly pitted. The combination used did not protect aluminum.

HOMOGENIZERS

This method has been found effective in maintaining homogenizers in a satisfactory condition. The machine should be cleaned by pumping lukewarm water through it to remove as much as possible of the milk or cream. It is then filled with the trisodium phosphate-chromate solution, the valves closed, and allowed to stand until it is used again. The solution is then drained out and the machine thoroughly flushed with hot water.

FACTORS AFFECTING THE ACTIVATABILITY[1] OF MILK WITH ULTRA-VIOLET LIGHT

W. E. KRAUSS, R. M. BETHKE, AND R. G. WASHBURN

Departments of Dairy and Animal Industry, Ohio Experiment Station, Wooster, Ohio

Supplee and coworkers (1–6) have made extensive and carefully controlled studies of the effect of various technical features of the irradiation process that might affect the ultimate vitamin D potency of milk. They have also investigated the influence of certain milk constituents, such as cholesterol and the percentage of fat, on activatability (7, 8).

It was our object to study activatability from the standpoint of factors that might be effective before the milk had reached the irradiation point. Breed, feed, and fat percentage were the factors studied. It was hoped that the data obtained would yield information as to the necessity for varying the conditions of irradiation to meet the peculiarities of milk production in a particular area. In spite of the fact that standardization of milk to a definite fat percentage is practiced to a considerable degree studies on the effect of fat percentage were included because of the association of vitamin D with fat.

PROCEDURE

The apparatus used in the irradiation process consisted of a carbon arc lamp[2] provided with a reflector which insured uniform distribution of the light on a thin film of milk flowing over one face of a small surface cooler having a total surface area of 3820 sq. cm. (59×65). The lamp was equipped with Industrial C carbons and was operated at approximately 50 volts and 60 amperes. The rate of flow of milk over the cooler, and consequently the thickness of the film, was determined by measuring at frequent intervals the volume of milk coming from the spout of the cooler in a given length of time and was maintained constant by adjusting a valve leading from the reservoir over the cooler and a valve leading from the milk source to the milk pump.[3] A recording ammeter made a visible continuous record of the amperage output and was supplemented by almost continuous observation of the ammeter in the control cabinet during the operation of the lamp.

By determining with a stop-watch the time required for a dye to traverse the cooler it was calculated that when the rate of flow was about 1000 cc. per minute the thickness of the film was 0.12 mm. Supplee and Dorcas (4) have shown that 95 per cent or more of the incident radiation below about

Received for publication June 29, 1936.

[1] By "activatability" of milk is meant the degree to which a given sample of milk becomes enriched with vitamin D when exposed to definite conditions of irradiation with ultra-violet light.

[2] Courtesy of the National Carbon Company, Cleveland, Ohio.

[3] Courtesy of the Creamery Package Manufacturing Co., Chicago, Illinois.

2850 Å is absorbed by the first 0.11 mm. depth. Since 960 cc. was the slowest rate of flow used it can be assumed that at least 95 per cent of the incident radiation was absorbed.

It should be pointed out, however, that in the work reported in this paper absolute measurements were superfluous; it was necessary only to maintain constant conditions when a particular set of samples was prepared. This was accomplished by treating the samples exactly alike up to the point of irradiation and by having the rate of flow, the distance from the arc, and the amperage output as nearly alike as physically possible for the immediate samples to be compared.

All the samples of milk used came from cows in the Experiment Station herd. This made it possible to control the feeding as desired and made available complete histories of the treatment, feeding, and production records of the animals. As a rule the samples represented the entire production of one or more cows over periods ranging from one to five days, depending on how much milk was needed. The amount of butterfat in the milk was determined. Standardized milk samples were prepared on the basis of the fat content of the cream and skimmilk. The percentage of fat in the standardized samples was also obtained.

To determine the relative vitamin D potencies the standard line test procedure with rats was followed. The test material was fed for 8 days and the rats were killed on the eleventh day, Steenbock and Black's rickets-producing ration 2965 being fed throughout.

In order to hold the samples in good condition for the length of time required in making the line test 6 to 8 drops of 40% formalin were added per quart; in a few instances the milk was dried before fans in a drying chamber. When dried milk was assayed at a high level it was incorporated with such a quantity of the basal ration as it was estimated would be consumed in five days; at low levels the assays were conducted as with liquid milk.

RESULTS

Effect of Fat Percentage

Three separate series were run on milks standardized to different fat percentages, using skimmilk and cream from an original sample of whole milk in each series. In Series I the milk came from three Holstein cows receiving a good winter ration. In Series II and III pooled milk from Jerseys and Holsteins was used, some of the milk in Series II coming from cows on pasture. All the milk in Series III was produced under winter feeding conditions. Details concerning the samples and the results of the line test are given in Table 1.

The standard winter ration of the Experiment Station dairy herd, from which all samples were obtained, consists of alfalfa hay, corn silage, corn, oats, bran, and linseed oilmeal, the grain being fed according to production.

TABLE 1

Effect of fat percentage on activatability of milk

CHARACTERISTICS OF SAMPLE		CONDITIONS OF IRRADIATION			CONDITIONS OF ASSAY		
No.	% Fat	Distance of arc from milk (inches)	Amperage	Rate of flow (cc./min.)	No. of rats	Total milk Fed (cc.)	Line test*
			Series I				
73a	1.90	20	60	960	5	16.0	0.90
					6	24.0	1.25
74a	5.85	20	60	960	6	16.0	1.83
					6	24.0	2.67
75a	0.025 (skimmilk)	20	60	960	5	40.0	0.40
					4	80.0	2.00
			Series II				
115x ...	0.03 (skimmilk)		Unir-radiated		9	64.0	0.20
115a ...	0.03 (skimmilk)	20	58	1380	9	64.0	0.56
115b ...	0.03 (skimmilk)	20	56	1860	9	64.0	0.61
116a ...	3.30	20	55	1380	9	16.0	0.79
117a ...	5.90	20	57.5	1200	9	16.0	1.40
117a ...	5.90	20	55	1800	9	16.0	1.30
			Series III				
119a ...	0.025 (skimmilk)	22	58	1125	8	64.0	0.20
120a ...	1.50	22	58	1120	8	24.0	0.20
121a ...	3.20	22	57.5	1110	7	20.0	0.36
122a ...	6.30	22	57	1130	6	20.0	1.10

* 1.00 equals unit healing (approximately 3.0 International Units)

When on regular pasture (mixed grasses) reduced quantities of the same grain mixture are fed.

The data show that in each series as the fat percentage increased the ability of the milk to become activated increased. This observation is more or less in agreement with that of Supplee, Flanigan, Bender, and Dorcas (7) who state that "the fat content does influence to a certain but limited degree the rate at which the antirachitic properties are imparted to the milk" but whose data show that under the same conditions of irradiation the vitamin D per cc. of milk increased as the fat percentage increased, levels of 0.6%, 1.2%, and 3.6% fat being used. The next level used by these investigators was 7.2% fat, at which point the vitamin D potency was no greater than at 3.6% fat under the same conditions of irradiation. Our data show a distinct increase in the potency of 6.3% milk over 3.2% milk. Supplee *et al.* also state

that "the *maximum*[4] antirachitic activity of milk resulting from direct irradiation does not parallel the fat content." It should be pointed out that this statement and our data are not contradictory since we were not concerned with maximum potencies.

EFFECT OF FEED

Winter Feeding vs. Pasture Feeding.—In a preliminary trial (Table 2, Series I) a sample of winter milk from one cow was compared with a pooled sample of pasture milk, both samples having approximately the same fat percentage. Although the unirradiated pasture milk was considerably more potent than the unirradiated winter milk, after irradiation the potencies of the two milks were approximately the same.

More significant data were obtained by collecting milk from the same cows while on winter feed and after they had access to spring bluegrass pasture for two weeks. The samples were dried so that they could be assayed simultaneously. This series also included a sample of milk from cows pasturing on wheat. The results (Table 2, Series II) indicate that irradiated pasture milks are slightly more potent than irradiated winter milks. Since the unirradiated pasture milks were more potent than the unirradiated winter milks it is probable that the original potencies were reflected in the irradiated milks. The evidence does not indicate the presence in pasture milks of any more activatable material than is present in winter milk.

High Protein Grain vs. Low Protein Grain.—Since there were available cows that had been fed for a long time on rations that were extremely low or extremely high in protein it was thought of interest to include the milk from these cows in this study. The rations differed not only in the amount of protein they furnished but in the vitamin D-carrying components as well. Alfalfa hay and a small amount of corn silage constituted the roughage of the high protein group, while timothy hay and a liberal quantity of corn silage made up the roughage of the low protein group. From Table 2, Series III, it will be seen that untreated milk from the high protein cows contained more vitamin D than untreated milk from the low protein cows. This undoubtedly was due to a greater intake of vitamin D from the alfalfa than from the timothy. After irradiation the milk from the high protein cows was more potent, reflecting again the original potencies of the untreated milks.

A.I.V. Silage vs. Alfalfa Hay.—One group of cows was fed alfalfa hay and one group A.I.V. (mineral acid treated) silage as the only roughage, the rest of the ration being the same for both groups. That the untreated milk from the A.I.V. cows was less potent than that from the alfalfa cows could have been anticipated because much more vitamin D had been found in the hay than in the A.I.V. silage. This relationship existed after irradiation (Table 2, Series IV). Because no positive line test response was obtained

[4] Authors' italics.

TABLE 2

Effect of feed on activatability of milk

CHARACTERISTICS OF SAMPLE			CONDITIONS OF IRRADIATION			CONDITIONS OF ASSAY		
No.	% Fat	Source	Distance of arc from milk (inches)	Amperage	Rate of flow (cc./min.)	No. of rats	Total milk fed	Line test*
			Series I					
97a	3.65	Winter feeding		Unirradiated		8	120 cc.	1.56
98a	3.65	" "	20	60	1050	8	16 cc.	1.70
99a	3.70	Pasture feeding		Unirradiated		8	120 cc.	2.90
100a	3.70	" "	20	60	1050	8	16 cc.	1.56
			Series II					
109b†	4.00	Winter feeding		Unirradiated		6	12 gm.	0.33
						8	200 mg.	0.30
109a†	4.00	" "	20	59	1320	8	300 mg.	0.90
118a†	4.00	Pasture feeding Same cows as 109b		Unirradiated		6	12 gm.	1.30
						7	200 mg.	0.60
118b†	4.00	" " " "	20	56	1320	7	300 mg.	1.00
114b†	4.30	Pasture feeding (Green wheat)		Unirradiated		6	12 gm.	1.20
						8	200 mg.	0.94
114a†	4.30	" "	20	60	1296	7	300 mg.	1.43

TABLE 2—(*Continued*)

CHARACTERISTICS OF SAMPLE			CONDITIONS OF IRRADIATION			CONDITIONS OF ASSAY		
No.	% Fat	Source	Distance of arc from milk (inches)	Amperage	Rate of flow (cc./min.)	No. of rats	Total milk fed	Line test*
			Series III					
123a	3.40	Cows fed high protein ration		Unirradiated		5	100 cc.	1.80
123b	3.40	" " " " "	20	60	1116	7	20 cc.	0.43
124a	3.20	Cows fed low protein ration		Unirradiated		5	100 cc.	0.40
124b	3.20	" " " " "	20	55	1110	7	20 cc.	0.00
			Series IV					
125a	3.95	Cows fed alfalfa as sole roughage		Unirradiated		7	80 cc.	0.57
						8	12 cc.	0.20
125b	3.95	" " " " " "	20	57	1122	8	16.8 cc.	0.94
126a	4.05	Cows fed A.I.V. as sole roughage		Unirradiated		7	80 cc.	0.21
						8	12 cc.	0.00
126b	4.05	" " " " " "	20	57	1140	8	16.8 cc.	0.00
127b	4.10	Cows fed alfalfa as sole roughage	20	57	1068	7	20.0 cc.	2.90
128b	4.05	Cows fed A.I.V. as sole roughage	20	57	1104	7	20.0 cc.	2.00

* 1.00 equals unit healing (approximately 3.0 International Units).

† Dried.

when irradiated A.I.V. milk was fed at 12.0 and 16.8 cc. levels the trial was repeated at a higher level on freshly collected batches of both milks, with the same results. The much greater line test response obtained on both irradiated milks after increasing the level of feeding but slightly might be attributed to the fact that new carbons were used in the lamp. This was the only change made so far as we are aware. The well-known variation in response of different groups of rats might also have exerted some influence.

So far as effect of feed is concerned, then, it seems that when the fat percentage is approximately constant the chief factor determining the vitamin D potency of milks irradiated under comparable conditions is the original potency of the unirradiated milks.

EFFECT OF BREED

Holstein milk and Jersey milk from cows on the same ration were separated and then standardized to 3.5% fat with the respective creams and skimmilks. Part of the standardized milks was irradiated and both unirradiated and irradiated samples were assayed for vitamin D. The standardized milks from both breeds had approximately the same vitamin D potency before and after irradiation (Table 3, Series I). If anything, the Jersey milk was slightly more potent before irradiation and the Holstein milk was slightly more potent after irradiation.

The next step consisted of assaying unstandardized Jersey and Holstein milks before and after irradiation (Table 3, Series II). In this case the unirradiated Jersey milk was definitely more potent than the Holstein unirradiated milk but after irradiation both milks had the same potency. A repetition of this trial yielded the same results (Table 3, Series III).

Previously (Table 1) it had been shown that milks containing 5.9% and 6.3% fat, respectively, were more potent after irradiating than milks containing 3.3% and 3.2% fat, respectively. The samples represented pooled milk from a mixed herd of Jerseys and Holsteins. In view of the results obtained with the breed milks (Table 3), the possibility is suggested that Holstein milk contains in the solids-not-fat fraction more activatable material than does Jersey milk.

DISCUSSION

The data presented lend support to the contention that in commercial irradiation of milk standardized to a definite fat percentage, milk of the desired vitamin D potency will be obtained so long as the conditions of irradiation are established on milk which was produced toward the end of the winter feeding period when its natural vitamin D potency was lowest. After the conditions of irradiation have been established on such milk the usual variation in the processes that influence the characteristics of milk up to the point of irradiation would probably be of insufficient magnitude to cause the milk to drop below the minimum required potency.

TABLE 3
Effect of breed on activatability of milk

CHARACTERISTICS OF SAMPLE			CONDITIONS OF IRRADIATION			CONDITIONS OF ASSAY		
No.	% Fat	Source	Distance of arc from milk (inches)	Amperage	Rate of flow (cc./min.)	No. of rats	Total milk fed (cc.)	Line test*
				Series I				
129a	3.5	Holstein milk (standardized)		Unirradiated		7	80.0	0.36
129b	3.5	" " "	20	57	1080	9	16.8	0.39
130a	3.5	Jersey milk (standardized)		Unirradiated		7	80.0	0.50
130b	3.5	" " "	20	57	1074	9	16.8	0.28
				Series II				
131a	3.15	Holstein milk		Unirradiated		7	100.0	0.70
131b	3.15	" "	18	55	1080	7	20.0	2.40
132a	5.25	Jersey milk		Unirradiated		7	100.0	1.60
132b	5.25	" "	18	54	1074	7	20.0	2.30
				Series III				
133a	3.25	Holstein milk		Unirradiated		8	80.0	0.50
133b	3.25	" "	20	57	1248	7	16.0	1.14
134a	5.40	Jersey milk		Unirradiated		8	80.0	0.75
134b	5.40	" "	20	57	1218	9	16.0	1.17

* 1.00 equals unit healing (approximately 3.0 International Units).

SUMMARY

Information was obtained as to the extent to which fat percentage, breed, and feed affected the ultimate vitamin D potency (activatability) of milk subjected to the same conditions of irradiation with ultra-violet light.

In general, as the fat percentage of the milk increased the activatability increased.

When the fat percentage is approximately constant the chief factor determining the vitamin D potency of milks irradiated under comparable conditions is the original potency of the unirradiated milks. This was determined by studying the activatability of milks produced under widely different systems of feeding in which the type of roughage was the principal variant.

Holstein and Jersey milks standardized to the same fat percentage were activated to the same degree on irradiation. Unstandardized milks from the same breeds were also activated to the same degree even though the Jersey milk contained appreciably more fat and possessed greater original vitamin D potency. This suggests that the solids-not-fat fraction of Holstein milk may contain more activatable material than that of Jersey milk.

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THE "TRANSITION POINT" OF MILK FAT

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If milk fat is an alloy-like mixture, then the cooling curve of a molten milk fat should show a definite break with the appearance of a second phase. Horn and Osol (1) in the case of cacao butter, using a cooling medium of melting ice, reported supercooling with a subsequent rise in temperature to a definite maximum. This maximum temperature they called the transition point. Horn and Wilson (2) later used this method to determine the degree of adulteration of cacao butter with milk fat.

In attempting to employ the above method to obtain a cooling curve for milk fat, it became apparent that the "transition point" was several degrees lower than the temperature where the second phase appeared as shown by visible turbidity. This was also apparent when the cooling medium was at a temperature of 20° C. It became evident, therefore, that if the transition point was to approximate the temperature of the appearance of the second phase, a much slower rate of cooling was necessary.

The following technique proved satisfactory. Thirty-five grams of melted fat at a temperature of 40–45° C. are carefully introduced into a pyrex, silvered, evacuated Dewar tube, 8 inches in length and 1 inch in diameter inside dimensions. This tube is fitted with a rubber stopper through which passes a thermometer graduated in 1/10 degrees, the bulb of which extends well down into the fat. The tube is also fitted with a small glass stirrer, the shaft passing through the stopper and the hollow circular base being such that it is free to move vertically without touching the sides of the tube or thermometer within it. The tube thus fitted is suspended in a water bath, the temperature of which may be regularly and progressively raised or lowered, or maintained constant, according to the desires of the operator. This temperature control within the temperature range here employed is very satisfactorily attained by the use of an adjustable-level water reservoir and a knife-edge 500 Watt heater in a closed circuit with the reservoir and the bath.

By maintaining a sufficiently small temperature gradient between the fat and the bath, very slow cooling is effected. Stirring should be restricted to not more than one up and down stroke per minute. During the initial cooling period the temperature may be read every five minutes, but as the temperature of the fat approaches the melting point reading should be taken every minute.

As long as the constant temperature gradient is maintained and the system consists of a single liquid phase, the rate of cooling remains uniform.

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As soon as a second phase appears, the rate of cooling becomes irregular, growing less and less until cooling ceases. The separation of the second phase is usually so slow that even with a small temperature gradient a certain amount of supercooling takes place. As the separation becomes sufficiently rapid the temperatures rises to a maximum. At this point—the so-called transition point—the temperature remains constant, within 0.01° C., for a period often as long as 30 minutes, even with a temperature gradient as large as one degree. This period is followed by a slow lowering of the temperature. The fat at this stage has the appearance of crystals suspended in a clear liquid. Duplicate determinations may be expected to correspond within 0.01 degree. No greater variation than 0.05 degree should be accepted.

The resulting time-temperature curves for a few samples of milk fat are shown in figure 1. These fats differ with respect not only to the transition points but also to the entire transition periods. They illustrate a variation which may be expected to exist between milk fats which have been secreted by cows subjected to a varying feeding regime. Curve U. F. represents a fat produced by a herd under spring feeding conditions, while curve X represents a fat produced under a dry feeding regime. Curves 4–323, 10–323, 12–323, 16–323, 26–323 and 28–323 represent a series of fats produced by cow 323 of the Station herd on the dates surrounding October 13, October 26, November 2, November 16, December 15 and January 12, respectively. Cow 323 was 8 years old and began the fifth month of her lactation period on October 9. On October 13 she was fed a ration of concentrate, corn silage, green alfalfa and pasture. Curve 4–323 is the cooling curve of the fat at that date. No. 10 fat was obtained immediately following the time when alfalfa hay was substituted for the remainder of the ration. For No. 12 fat the cow was supposedly on the ration of alfalfa hay, but investigation revealed her to have had access to green feed the preceding day. Curves 16 and 26 represent fats obtained while the cow was still on the ration restricted to alfalfa hay. On December 22 corn silage was added to the ration. Curve 28–323 represents the fat after an interval of about 3 weeks.

Special mention should be made of curve R. This fat was produced by a cow which was establishing a world's record for fat production in her class. The fat yield was out of proportion to the feed consumed. Chemical analyses of this fat, as well as the physical condition of the cow indicated that body fat was being utilized in the production of milk fat. At 37.5° the fat was clear, while at 36.4 a turbidity was visible. This latter increased as the temperature was gradually lowered. The appearance of turbidity coincided almost exactly with the first change in the direction of the curve. No supercooling with subsequent rise to a maximium took place. Unfortunately, not sufficient of this fat was available to permit a thermal study at higher temperatures.

In contrast to milk fat, the cooling curve of a sample of a nut margarine was smooth and regular to a temperature of 22.5° C., when a second phase appeared, supercooling took place and this was followed by an increase in temperature to a maximum of 23.10° C. which persisted for 30–35 minutes.

DISCUSSION

Although Arup (3) subjected milk fat to fractional crystallization and analyzed fractions which separated at 37°, 27°, 20°, 15° C, as well as those which were liquid at 15 and 10° C., yet milk fat has not been subjected to extensive thermal analysis. Rahn (4) has shown that the congealing point of milk fat varies with the temperature of the cooling bath. His cooling curves from progressive cooling showed a maximum rise in temperature at the congealing point (22.5–24° C.) with a fairly smooth curve down to 12–14° C. The nature of the cooling curves of samples of cacao butter has been reported by Pichard (5) and by Straub and Malotaux (6). Each observed typical supercooling with a rise to a maximum which was called the setting or congealing point. The "transition point" of Horn and Osol (1) was several degrees higher for cacao butter than the "setting point" of Pichard, Straub and Malotaux.

Theoretically the temperatures of complete fusion and incipient solidification should coincide. In other words, fusion and solidification are two reciprocal and reversible phenomena. In the case of végétaline and stearine Chatelier and Cavaignac (7) confirmed such a reversibility but pointed out that the point of fusion approaches much nearer than the point of solidification to the exact temperature of the change of state. This latter may be accounted for on the assumption that supercooling is more prevalent than superheating.

Milk fat is a somewhat more highly complex alloy-like system than the natural vegetable fats. Consequently, a more highly refined technique than that used by Chatelier and Cavaignac is essential to confirm the reversibility. In figure 1 the values for melting points and transition points differ by 1.3 to 3.7 degrees. These differences should not be interpreted to mean that the temperatures of complete fusion and incipient solidification are not coincident. In making melting point determinations of milk fat by the Wiley method the author has considerable difficulty in determining when the disc of fat has assumed a spherical shape and has become definitely clear. With some milk fats it often happens that a difference of as much as two or three degrees lies between the temperature when the fat appears practically clear and the temperature when it may be judged absolutely clear. In the case of cacao butter, Horn and Osol (1) found as great a difference as 6° between the temperatures of incipient fusion and complete fusion. These considerations together with the acknowledgment that the melting point values were secured for the purpose of comparison between the fats rather than for precision purposes permit of the assumption that the transition

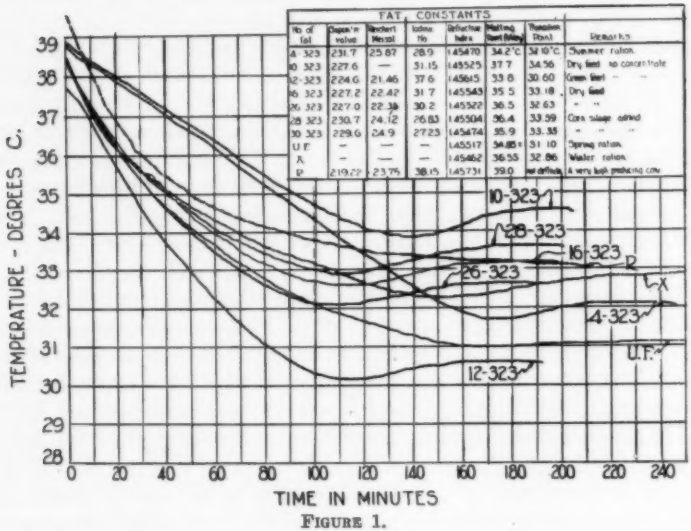

FIGURE 1.

point determined by this refined technique represents a temperature more nearly coincident with the true fusion temperature than does the melting point by the methods generally accepted. Its precision compensates for its tediousness.

CONCLUSIONS

1. The cooling curve of milk yields an insight into the physical and chemical nature of the fat.

2. The "transition point" when determined by the modified technique represents a temperature where a second phase begins to separate from a molten fat.

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OXIDIZED FLAVOR IN MILK

III. THE TIME OF COPPER CONTAMINATION DURING PRODUCTION AND PROCESSING, AND AERATION VERSUS NO AERATION AS RELATED TO OXIDIZED FLAVOR DEVELOPMENT*

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Various investigators have studied conditions necessary for the development of oxidized flavor, but little attention has been directed to the relationship between the time of contamination with copper and the pasteurizing process. Likewise little is known concerning the effects of surface and internal cooling on the development of oxidized flavor in milk.

In order to determine whether pasteurization has any effect on the development of oxidized flavor in milk it was decided to study this factor by the use of specially constructed glass pasteurizing and cooling equipment, in which milk could be handled under conditions similar to those obtaining in milk-pasteurizing plants, with the exception that no metallic contamination of the milk by the equipment would be possible.

EQUIPMENT

The glass pasteurizing equipment consisted of a five-liter round-bottom flask held in position in a water bath by means of a suitable clamp. A motor-driven agitator, made of glass, was operated in the milk at such a speed as to cause gentle agitation of the milk. The speed of the agitator was controlled by operating it on a belt from a motor with a variable speed drive through a reduction pulley. A second agitator, operated in the water bath, served to bring about a uniform distribution of heat throughout the water bath. The water bath was heated by means of flowing steam and the level of the water in the bath was controlled by the introduction of water through a rubber hose from a tap, and by removal through a siphon. Thermometers suitably clamped in position served as guides for the manual control of the temperature of the milk. With these arrangements, and with the constant attention of one person, the pasteurizing temperature could be controlled within one degree of that desired. Figure 1 illustrates this assemly.

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The data presented herein are to be included in a thesis by W. Carson Brown in partial fulfillment of the requirements for the degree of Doctor of Philosophy, West Virginia University.

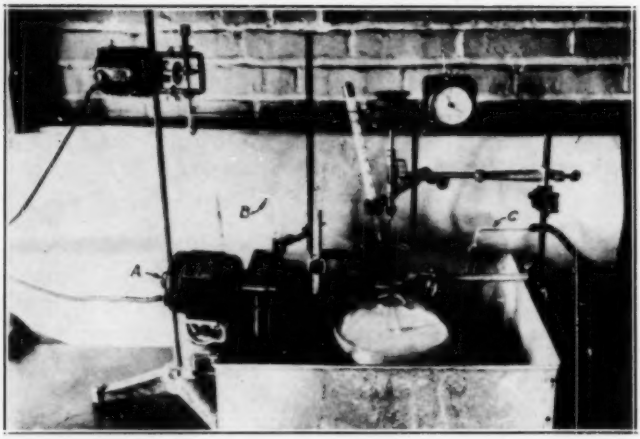

FIG. 1. EQUIPMENT USED FOR PASTEURIZING MILK IN GLASS
A, motor-driven water agitator; B, siphon for controlling water level; C, steam inlet. Purpose of other equipment is obvious.

The glass cooling equipment consisted of two units, one for surface cooling and the other for internal cooling. Both units were mounted on a specially constructed stand as illustrated in Figure 2. The equipment for internal cooling consisted of a condenser 30 inches long with the inner tube similar to Vigreaux distilling tube. Ice water from an elevated ice cream packing tub, which was charged with ice and water, served as the cooling medium. When used as a cooler the condenser was filled with milk and a volume of the milk equal to the capacity of the condenser was withdrawn while keeping the condenser filled with milk before experimental samples were taken. In this way exposure of the milk to air was kept at a minimum for the equipment used.

The equipment for surface cooling consisted of a series of 21 glass tubes (10 mm. diameter), bent upward at an angle from points near each end, and fastened into a wooden frame. The bends in the tubes served to prevent any flow of milk against the wooden frame. Alternating with each tube in the frame was a piece of flat window glass two inches wide through its center and tapered at the ends to fit the bends in the tube below it. The flat glass served to increase the aerating surface and also to aid in directing the flow of milk over each tube. The upper 11 tubes of the cooler were connected with rubber tubes. Tap water, circulated through these tubes from bottom to top served as the cooling medium. The lower ten tubes also were connected with rubber tubes, and ice water from the elevated ice cream packing tub was circulated through this section of the cooler. The total length of this cooler was about

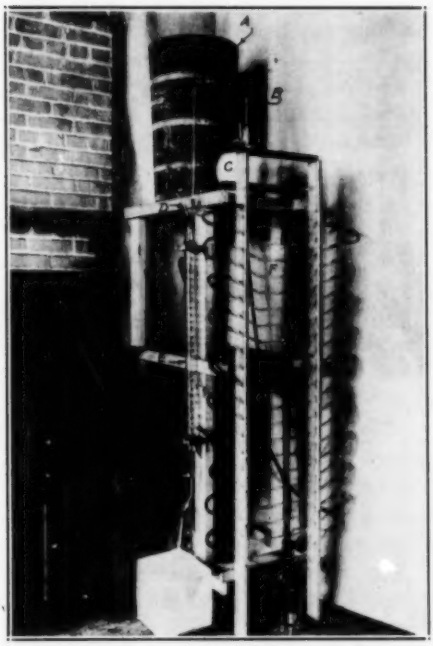

FIG. 2. EQUIPMENT USED FOR COOLING MILK IN CONTACT WITH GLASS
A, Ice cream tub containing ice and water; B, siphon; C, flask containing hot milk; D, three-way stopcock; E, internal cooler; F, surface cooler.

4.5 feet. Milk was delivered to the cooler through a removable glass tube fire-sealed at one end and having a series of small holes in its upper surface.

When preparing to cool milk a special glass siphon was placed in the flask containing the hot, pasteurized milk and the flask then was clamped into position on the wooden frame above the two coolers. The siphon was equipped with a three-way stopcock so that milk could be delivered either to the surface cooler or to the internal cooler.

Other equipment used in this work does not require description.

EXPERIMENTAL

Experiments Using the Special Glass Equipment

All the milk used in these experiments was from cows selected because their milk was known to be susceptible to oxidized flavor development. The milk was drawn into aluminum pails and poured directly into aluminum cans, omitting the usual straining operation because the strainers available at the time this work was done exposed copper surfaces which came in contact

with the milk that was passed through them. The milk was brought to the laboratory immediately and cooled by immersing the cans in ice water.

Samples of the raw milk in pint milk bottles were prepared containing 0.5, 1.0, 1.5, 2.0, and 2.5 parts per million of added copper,[3] respectively. One sample to which no copper was added served as a control in each trial. The remaining milk, in each trial, was used in studies of the effect of pasteurization. Each pasteurization was carried out at 143° F $\pm$ 1° for 30 minutes. Before being used the surface cooler was washed with alkaline solution and rinsed with distilled water. Pasteurizations in this experiment were carried out in two series, one in which copper was added before pasteurization, and the other in which copper was added after pasteurization and cooling. The amounts of copper added in each series were the same as those used in the raw milk, previously detailed, and part of each milk in each series was surface-cooled, whereas the remainder was cooled internally. The milk usually was at a temperature of 50 to 55° F. after internal- or surface-cooling, and accordingly all samples were immersed in ice water for several hours as soon as they were prepared.

To avoid, in so far as possible, any effect that light might have during processing and storage, all pasteurizing and sampling operations were carried out at night under illumination from red light-bulbs, and the individual samples in pint milk bottles, when removed from the ice water, were placed in brown paper bags during storage for three days at approximately 40° F. At the end of the three-day storage period the samples were tasted and the intensity of the oxidized flavor was recorded.

The results of this study are recorded in tables 1 and 2. Table 1 shows a comparison of the effects of internal and surface cooling, and table 2 shows the effects of adding copper before pasteurization as compared to adding it after pasteurization.

Studies With the Milk of Individual Cows

The results recorded in table 2 indicate that there is a decided, though not entirely consistent, tendency for oxidized flavor to develop more readily, and to a greater intensity, in milks to which copper was added after pasteurization than in milks to which copper was added before pasteurization. In order to obtain further information on this point the milk from each cow in the Experiment Station herd was studied individually. The milk was drawn into aluminum buckets and several pint milk bottles were filled immediately with the milk of each cow. Raw-milk samples, with and without added copper, were stored for three days at 40° F. before tasting. The re-

[3] Copper sulphate solution was added. In preparing the solution crystals were brushed free of any anhydrous salt that may have been present, and the solution was made up to a strength such that not more than 2.5 ml. was required in a pint of milk to make the added copper amount to 2.5 parts per million.

TABLE 1

Comparison of internal cooling with surface cooling of milk in relation to development of oxidized flavor. (Copper added after pasteurization)

TREATMENT	COPPER ADDED PPM.	TRIAL NUMBER				AVERAGE INTENSITY
		I	II	III	IV	
Internal Cooling	None	–*	–	–	–	–
	0.5	1	1	3	2	1.75
	1.0	2	2	4	2 to 3	2.63
	1.5	3	2	3	3 to 4	2.88
	2.0	3	2	3	4	3.0
	2.5	3	2	3 to 4	3	2.88
Surface Cooling	None	–	–	–	–	
	0.5	1	1	3	3	2.0
	1.0	1	2	3	2 to 3	2.13
	1.5	2	4	3	lost	3.0
	2.0	1	4	4	4	3.25
	2.5	2	4	4	3	3.25

* Meaning of symbols: –, no oxidized flavor; ?, may or may not have been oxidized; 1, very slight oxidized flavor; 2, slight oxidized flavor; 3, moderate oxidized flavor; 4, fairly pronounced oxidized flavor; 5, pronounced oxidized flavor; and 6, very pronounced oxidized flavor.

TABLE 2

Relation of incidence of contamination with Cu to the development of oxidized flavor in raw and pasteurized milk

TREATMENT	COPPER ADDED PPM.	TRIAL NUMBER				AVERAGE INTENSITY
		I	II	III	IV	
Raw milk	None	–	–	–	–	–
	0.5	1	1	3 to 4	2	1.88
	1.0	3	1	3	3	2.50
	1.5	3	2	3	2	2.50
	2.0	3	2	3 to 4	2 to 3	2.75
	2.5	4	3	4	3	3.50
Pasteurized in glass container (Copper added *before* pasteurizing)	None	–	–	–	–	–
	0.5	?	?	1	2	0.75
	1.0	?	?	?	1	0.25
	1.5	?	?	?	1	0.25
	2.0	?	?	1	2	0.75
	2.5	Missed	?	1	3	1.00
Pasteurized in glass container (Copper added *after* pasteurizing)	None	–	–	–	–	–
	0.5	1	1	3	2	1.75
	1.0	2	2	4	2 to 3	2.63
	1.5	3	2	3	3 to 4	2.88
	2.0	3	2	3	4	3.00
	2.5	3	2	3 to 4	3	2.88

For meaning of symbols in above table see footnotes, Table 1.

TABLE 3

The relation of pasteurization and of the addition of copper before or after pasteurization to the subsequent development of oxidized flavor in the milks of individual cows. Copper added to all milks at the rate of 1.3 p.p.m.

DATE	TOTAL NUMBER OF COWS TESTED	RAW MILKS HAVING OXIDIZED FLAVOR WITH ADDED COPPER		PASTEURIZED MILKS HAVING OXIDIZED FLAVOR			
				Copper added before pasteurization		Copper added after pasteurization	
		Number of cows	Per cent of cows tested	Number of cows	Per cent of cows tested	Number of cows	Per cent of cows tested
4/ 6/36	44	16	36.4	8	18.2	26	59.1
4/12/36	44	10	22.7	2	4.5	19	43.2
4/20/36	40	15	37.5	9	22.5	26	65.0

TABLE 4

The effects of the addition of 1.3 parts per million of copper before and after pasteurization to samples of the milks of individual cows. (Samples taken 4/20/36)

COW NO.	NO COPPER ADDED	COPPER ADDED *before* PASTEURIZATION	COPPER ADDED *after* PASTEURIZATION
176	–	–	3
305	–	1	2
319	–	–	1
321	–	1	3
326	–	2	2
332	–	–	2
336	–	2	3
347	–	–	2
358	–	2	2
359	–	–	1
360	–	–	3
361	–	–	3
362	–	2	3
363	–	–	3
366	–	–	2
367	–	–	1
368	–	–	2
369	–	–	?
373	–	2	2
381	–	–	2
388	–	1	3
389	–	–	3
390	–	3	3
391	–	–	1
392	–	–	2
395	–	–	2
398	–	–	1
405	–	–	?

* Records of all cows producing milk that was not susceptible to oxidized flavor development were omitted from table. For meaning of symbols see Table 1, footnotes.

maining samples were pasteurized for 30 minutes at as near 143° F. as possible by immersion in a hot-water bath, one sample from each cow having copper added to it before pasteurization and another afterward. The samples were cooled, then stored and tasted along with the raw-milk samples. All additions of copper in this experiment were at the rate of 1.3 parts per million. The experiment was repeated twice.

A summary of the results of these experiments is shown in table 3, and a detailed tabulation of the results of one of the experiments is recorded in table 4.

DISCUSSION

Surface cooling with the resulting exposure of the milk in a thin film to the air might be expected to favor the development of oxidized flavor, especially as compared to internal cooling. This appears not to be the case as shown by these experiments. It would appear from these results that only surface coolers from which the milk may dissolve copper would be likely to contribute to oxidized flavor development.

So far as the authors are aware, it has not been observed previously that contamination of susceptible milk with copper *after* pasteurization tends to cause a more pronounced development of oxidized flavor than does contamination with copper *before* pasteurization. This effect seems to obtain as shown by the experiments herein reported.

The effect on oxidized flavor development of the time of copper addition or contamination in relation to the time of pasteurization has both a practical and an experimental application. From a practical standpoint it would appear that the prevention of contamination of pasteurized milk with copper in the milk plant is of greater importance than the prevention of copper contamination of raw milk at the farm. It would seem also that slight contamination of the milk with copper at the farm would not result in the production of an oxidized flavor sufficiently intense to be objected to by many customers buying the milk after it has been pasteurized, provided no contamination with copper occurred during processing in the pasteurizing plant. However, no data on the effects of excessive copper contamination were obtained. From an experimental standpoint, in testing the susceptibility of milk to oxidized flavor development by adding known amounts of copper, more reliable results may be expected with raw milk or with pasteurized milk to which the copper has been added *after* pasteurization than with pasteurized milk to which the copper has been added *before* pasteurization.

At the present time there appears to be no satisfactory explanation for the effect of the time of adding copper in relation to the time of pasteurization on the development of oxidized flavor.

In this study comparisons of the development of oxidized flavor in pasteurized milk with that in raw milk do not yield entirely consistent results.

In table 2 the intensities of oxidized flavor developed in the raw milks in the four trials average almost exactly the same as the intensities for the pasteurized milks to which the copper was added *after* pasteurization. Results shown in table 3 are tabulated in terms of frequency of the occurrence of oxidized flavor rather than of intensity of this flavor, but the fact that in these results the flavor occurred less frequently in the raw milks than in the corresponding milks to which the copper was added *after* pasteurization would indicate that pasteurized milk is more susceptible to oxidized flavor development than raw milk. Of course it must be kept in mind that the results in table 3 are for the milks of individual cows whereas the results in table 2 are for mixed milks, and also that pasteurization and cooling of milks studied in connection with the results in table 3 were carried out by the in-the-bottle method whereas batch pasteurization and separate cooling were employed in the case of milks studied in connection with the results in table 2. However, these facts do not offer any explanation of the effects observed. It would seem that the only observation that should be made on this point at present is that when amounts of copper ranging up to 2.5 parts per million are added to susceptible milk the intensity of the resulting oxidized flavor may be expected to be greater in the raw milk than in the corresponding pasteurized milk when the copper contamination occurs *before* pasteurization, but may be expected to be about the same as that of the raw milk when the copper contamination occurs *after* pasteurization.

CONCLUSIONS

When pasteurization is carried out by the so-called low-long method contamination of the milk *after* pasteurization with amounts of copper ranging up to 2.5 parts per million tends to cause a more frequent and more intense development of oxidized flavor than does contamination with identical amounts of copper *before* pasteurization.

Raw milk, when contaminated with the above-stated amounts of copper, tends to develop oxidized flavor of about the same intensity as occurs in the same milk contaminated with identical amounts of copper *after* pasteurization, but of a greater intensity than occurs in the same milk contaminated with identical amounts of copper *before* pasteurization.

The exposure of milk to the air while being passed over a surface cooler does not, *per se*, cause any greater development of oxidized flavor than does the passage of milk through an internal cooler.

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AMYLASE IN COW'S MILK

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Recent workers (1, 2), interpreting Kuhn's (3) findings, apparently have accepted the assumption that α-amylase catalyzes the hydrolysis of central glucosidic linkages in the amylose molecule, thus accelerating starch dextrinization, with little or no accompanying saccharification. It has also been assumed that β-amylase induces saccharification without dextrinization, or at least considerable amounts of reducing material are produced before the blue coloration of the starch by iodine has disappeared. If this assumption is correct, then one may accept the evidence of the presence of α-amylase (dextrinizing-amylase) in cow's milk.

Sato (4) found that 100 ml. of raw cow's milk incubated for 24 hours at 40° C. converted 36 mg. of soluble potato starch into a form which no longer yielded the blue color with iodine. Likewise Chrzaszcz and Goralowna (5–6) by the starch-iodine method (using both raw and soluble starch) found that amylase is present in all samples of raw milk, its concentration being dependent upon the richness in fat, the stage of milking (foremilk being less rich in amylase than the strippings), the feed of the cow, the age of the cow and the health of the udder. These workers found that 100 ml. of normal milk could dextrinize 0.05–0.1 g. of soluble starch in 60 minutes at 30° C. In contrast to these findings, Manicatide, Bratescu and Popa (7) using a modification of the Wohlgemuth Method found that the great majority of samples of cow's milk contain no amylase, that a few samples contain from 2 to 4 Wohlgemuth units per ml. in contrast to human milk samples which contain from 128 to 512 units per ml.

Chrzaszcz and Goralowna (5), apparently the only workers to report a saccharifying enzyme in cow's milk, did not state the source of the milk samples, failed to use an antiseptic agent during incubation and made no mention of precautions taken to assure the milk samples were from normal, healthy udders. Kuttner and Somogyi (8), studying the saccharogenic power of cow's milk by employing pure corn or rice starch, reported amylase entirely absent. Their results are vitiated somewhat by the facts that soluble starch is the accepted substrate in such studies and that too minute amounts of milk were employed especially for so short an incubation period as 30 minutes.

This evidence of the incompleteness of knowledge concerning the presence of a true amylase in cow's milk suggested the desirability of undertaking a further study using the following criteria:

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(1) The starch-liquefying power of milk as measured by viscosity changes.
(2) The dextrinizing power of milk according to starch-iodine reactions.
(3) The saccharogenic power of milk as determined by increase in reducing power.

EXPERIMENTAL

(1) *Starch Substrates.* All starch substrates, except one, were 1.5%, prepared according to the method of Jozsa and Johnston (9). One soluble starch solution was prepared according to Gould (10).

(2) *Source of Milk.* The raw market milk supply of the Univeristy Farm Creamery furnished the majority of the mixed milk samples for the experimental work. When freshly drawn milk from individual cows was desired, samples were drawn aseptically into toluene from cows of known history. The diagnosis of mastitis was made according to methods outlined by Plastridge, Anderson, White and Rettger (11).

(3) *Milk Sera.* The lead sera were prepared by adding 3.0 ml. basic lead acetate solution (sp. g. 1.2) to 100 ml. of milk with intermittent shaking, and subsequent filtering. In those samples which were drawn aseptically into toluene and where bacteria were to be eliminated, the lead serum was filtered through L3 Pasteur-Chamberland filters at a reduced pressure of 15 inches, sterile apparatus being used. Precautions against the introduction of saliva were taken by the use of cotton-plugged pipettes.

Iron sera were prepared by the addition of 15 ml. of a 1.5 per cent solution of dialyzed colloidal iron to 5 ml. of milk diluted with 15 ml. of water, with vigorous shaking, and subsequent filtering. In those experiments where bacteria were to be eliminated the filtrates were again filtered through the Pasteur-Chamberland filters.

(4) *Procedure for measuring starch liquefying power.* The reaction mixture of milk, or milk serum, and freshly prepared raw potato starch, was placed in an Ostwald pipette, with outflow time of approximately 100 seconds for distilled water at 20° C. The pipette was placed in a water bath maintained at 37 ± 0.1° C. Boiled controls were used in every case. Inasmuch as the two ingredients were tempered in the bath prior to mixing, the outflow time of the mixture could be taken immediately upon being placed in the pipette. Outflow time was again recorded at the end of 1 hour.

(5) *Procedure for determining starch dextrinization.* To a series of test tubes containing increasing amounts of starch were added 10 ml. of milk and 0.3 ml. toluene. After thorough shaking they were incubated at 37° C. for 1, 5, and 24 hours. At the conclusion of the respective incubation periods 1 ml. of 0.033 N iodine-potassium iodide solutions (prepared according to Gould (10)) was added to each tube and the resultant colors recorded. Soluble potato starch and corn starch were compared using raw milk; raw and

pasteurized (61.5° C. for 30 minutes) milks were compared using soluble starch. In some trials lead or iron sera were prepared from the incubated samples, and to each was added 1 drop of 0.033 N iodine-potassium iodide solution, and the colors recorded.

(6) *Procedure for measuring starch saccharification.* Reaction mixtures consisting of 100 ml. of milk, 30 ml. of starch, and 5 ml. toluene were incubated at 37° C. for various intervals. Reducing sugar determinations were made before and after incubation using the method of Cole (12) and the percentage increases in reducing material were calculated.

RESULTS

Liquefaction. Ten samples of milk were employed to compare the decrease in viscosity of potato starch—raw milk (or milk serum) mixtures and potato starch—boiled milk (or boiled milk sera) mixtures. Table I shows that in all cases the decrease in viscosity at the end of one hour's incubation was appreciably greater in the case of the raw milk than in the boiled control.

TABLE I

Liquefaction of potato starch at 37° C. by milk

SAMPLE NUMBER	DECREASE IN VISCOSITY IN ONE HOUR	DECREASE IN VISCOSITY OF BOILED CONTROL IN ONE HOUR	NET DECREASE IN VISCOSITY IN ONE HOUR	RATIO OF ENZYME PREPARATION TO STARCH	HISTORY OF ENZYME PREPARATION
	per cent	*per cent*	*per cent*		
1	28.0	11.7	16.3	1:1	Fresh raw market milk
2	29.9	11.7	18.2	1:1	" " " "
3	36.9	9.4	27.5	1:1	" " " "
4	21.7	9.7	12.0	1:10	" " " "
5	24.1	9.9	14.2	1:10	" " " "
6	22.9	5.4	17.5	1:10	" " " "
7	7.5	4.0	3.5	1:10	Serum from raw market milk
8	9.6	4.0	5.6	1:10	Serum from milk of Cow No. 174*
9	7.6	4.0	3.6	1:10	Serum from milk of Cow No. 191*
10	8.0	4.0	4.0	1:10	Serum from milk of Cow No. 462*

* Lead acetate serum filtered through L3 Pasteur-Chamberland filter; sterile equipment used.

TABLE II

Starch dextrinization by milk

SAMPLE	STARCH	INCUBATED AT 37° C.	STARCH–IODINE COLOR CC. 1.5% STARCH ADDED TO 10 CC. MILK													
			0	0.05	0.10	0.15	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
		hours														
1. Fresh raw milk	soluble	1					Y*	Y	Y	B*	B	B				
" " "	corn	1	Y	Y	B	B	B									
2. Fresh raw milk	soluble	1					Y	Y	B	B	B	B	B	B	B	B
" " "	"	5					Y	Y	Y	B	B	B	B	B	B	B
" " "	"	24					Y	Y	Y	Y	Y	Y	Y	Y	B	B
3. Fresh pasteurized milk (61.5° C. for 30′)	soluble	1	Y	B	B	B	B									
"	"	5	Y	B	B	B	B									
"	"	24	Y	Y	Y	B	B									

* Y—Yellow.
B—Blue.

No attempt was made to determine the liquefon value (9) of milk, but it is apparent that considerable variation exists between milks from various sources. The lower values obtained with the lead sera may be partially explained on the basis of lead toxicity and partially on the possibility of amylase adsorption on the precipitated milk solids. The milks for samples 8, 9, and 10 were taken aseptically into toluene, the lead sera obtained, and these filtered through the Pasteur-Chamberland filters. The results indicate that the agent of liquefaction was not elaborated by bacteria during incubation. It is of interest to note that sample No. 8 was positive to the tests for chronic mastitis.

Dextrinization. Table II substantiates the findings of several investigators (Sato (4), Chrzaszcz and Goralowna (5)), that milk is able to dextrinize soluble starch. Soluble starch undergoes conversion more readily than corn starch and pasteurization at 61.5° C. (142.5° F.) for 30 minutes serves to retard the enzyme action, although it is not entirely destroyed. The milk samples studied were obtained from a commercial milk supply.

Saccharification. In view of the relatively high concentration of lactose in milk and the low concentration of amylase no attempt was made to isolate maltose from the incubated samples. It seemed justifiable to assume that an increase in reducing power of the mixtures would be due to maltose formation according to Freeman and Hopkins (13). Table III shows a progressive

TABLE III

Saccharification of starch by milk

INCUBATED AT 37° C.	SOLUBLE C. P.			RAW POTATO			RAW CORN		
	Reducing Material*		Increase	Reducing Material		Increase	Reducing Material		Increase
hours	*ml.*	*per cent*	*per cent*	*ml.*	*per cent*	*per cent*	*ml.*	*per cent*	*per cent*
0	5.27	4.43	—	5.00	4.64	—	5.27	4.43	—
5	5.08	4.59	.16	4.97	4.67	.03	5.10	4.58	.15
24	5.00	4.66	.23	4.93	4.71	.07	5.10	4.58	.15
Iodine color after 24 hours			yellow			blue			blue

* Calculated as lactose hydrate.

increase in reducing substance with increasing duration of incubation in the case of both soluble starch and raw potato starch with a tendency to reach a maximum in the case of corn starch. The color reaction with iodine at the end of the 24 hour incubation period was yellow with the soluble starch, and blue with the raw potato and corn starches. The color with iodine at the end of the incubation period for a given concentration of amylase depends upon the kind of starch used, the concentration of the starch and the length of the

TABLE IV

Amylase in milk sera filtered through the L3 Pasteur-Chamberland filter

SERIES NO.	TREATMENT OF SERUM	SOLUBLE STARCH SUBSTRATE	RATIO OF STARCH TO SERUM	REDUCING MATERIAL			INCREASE IN REDUCING MATERIAL	IODINE COLOR
				Before incubation	Incubated at 37° C. for 24 hours	Decrease		
				ml.	*ml.*	*ml.*	*per cent**	
1	Lead, diluted 1–7, unfiltered	Jozsa and Johnston 1.5%	1 : 10	4.12	3.98	0.14	0.14	Deep blue
	Lead, diluted 1–7, filtered	" "	"	4.12	4.00	0.12	0.12	Deep blue
	Lead, diluted 1–7, unfiltered	Gould, 1%	"	4.12	3.92	0.20	0.20	Red-blue
	Lead, diluted 1–7, filtered	" "	"	4.12	3.95	0.17	0.17	Red-blue
2	Lead, concentrated, filtered	Gould, 1%	1 : 5	4.02	3.85	0.17	0.18	Red
	Lead, concentrated, filtered	" "	2 : 5	4.05	3.87	0.18	0.19	Blue
	Lead, concentrated, filtered	" "	3 : 5	4.12	3.95	0.17	0.17	Blue
3	Iron, dilute, filtered	Gould, 1%	1 : 20	5.08	4.92	0.16	0.13	Light blue
	" " "	" "	1 : 10	5.25	5.07	0.18	0.13	Deep blue
4	Iron, dilute, filtered	Jozsa and Johnston 1.5%	1 : 20	4.00	3.87	0.13	0.14	Deep blue
	Fresh raw market milk	" "	3 : 10	3.93	3.80	0.13	0.14	Red-blue

* Calculated as lactose hyydrate.

incubation period. The temperature of incubation, the pH of the reacting mixture, and the presence of activators are other factors which must be considered if quantitative values are to be determined.

That the presence of amylase in milk is not due to bacterial activity is illustrated in Table IV. The milk samples were drawn aseptically into toluene, and the lead or iron sera were procured. Portions of these sera were filtered through Pasteur-Chamberland filters and the various mixtures of sera and starch were incubated at 37° C. for 24 hours. Amylytic activity is shown by increased reducing values of the mixtures at the end of incubation, amounting to from 0.13 to 0.20 per cent sugar. The concentration of the starch was such that dextrinization was never complete at the end of incubation.

Heat stability. The amylase activity of milk has been utilized to detect the improper pasteurization of milk or the presence of raw in pasteurized milk (10, 14, 15). It has been reported (16) that α-amylase is less resistant to heating than β-amylase, although Kuhn (3) did not find it necessary to postulate differences in heat stability. The following study was designed to determine the effect of heating milk at various temperatures on both the dextrinizing and saccharifying amylases of milk as well as to demonstrate further the presence of both α- and β-amylases in milk.

Raw market milk was heated in flasks in water baths at 50°, 55°, 60°, 65° and 70° C. for 30 minutes. One sample was flash heated at 90° C. They were then cooled to room temperature in ice-water, and reducing values determined. Mixtures of these milks with starch (20 ml. milk, 6 ml. of 1.5% soluble starch, 1 ml. toluene) were incubated at 37° C. for 24 hours. Reducing values of the mixtures were made, and the reactions of the lead sera with iodine were observed. A milk-toluene mixture was used as a control.

As shown in Table V, no significant difference in saccharogenic activity was found between raw milk and the milk heated for 30 minutes at 50, 55, 60, or 65° C. Milk heated at 70° C. for 30 minutes, or flash heated to 90° lost practically all of its power to increase the reducing value of the starch—milk substrate. In contrast, the starch dextrinizing activity of milk was adversely affected by the various heat treatments at temperatures of 55° C. and higher. From this it is inferred that starch saccharifying and starch dextrinizing enzymes are present in milk, and that the former is much less heat labile than the latter.

Effect of Incubation Temperature on Saccharifying Power. Rumsey (17) reported that the saccharifying amylase of flour exhibited maximum activity in the pH zone of 4.0–5.3 and with increasing temperatures up to 55° C. Chrzaszcz and Goralowna (5) found that normal milk showed an optimum dextrinizing activity at pH 5.8 to 6.2 at 30° C. The temperature of 30° C. was also found to be optimum for saccharifying action. The data shown in Table VI agree only in part with the latter findings. Raw market milk was

TABLE V

Heat Lability of Milk Amylase

TREATMENT	SUGAR DETERMINATIONS			IODINE TEST
	Sugar solution	Decrease	Increased reducing material	1 cc. .001N I–KI per 5 cc. serum
	ml.	*ml.*	*per cent*	
Raw (before incubation)	5.17	—	—	Blue
Incubated 24 hrs. at 37° C.:				
Raw	4.83	0.34	0.30	Yellow
50° for 30 minutes	4.82	0.35	0.31	Yellow
55° " " "	4.85	0.32	0.29	Blue
60° " " "	4.83	0.34	0.30	Blue
65° " " "	4.80	0.37	0.33	Blue
70° " " "	5.10	0.07	0.06	Blue
90° (flash)	5.15	0.02	0.02	Blue

* Calculated as Lactose hydrate.

used, and mixtures prepared in the ratio of 10 of milk, 3 of 1.5% soluble starch and 1 of toluene. The lead and iron sera were prepared from this mixture in the manner already described. Series of six tubes from each type of mixture were incubated at the temperatures indicated, the water lost through evaporation being replaced before final determinations were made. Milk and milk sera containing no starch when incubated at 30° C. and 70° C. for 24 hours showed no change in reducing value.

The optimum incubation temperature for saccharification is obviously in the vicinity of 50° C., but for dextrinization the optimum temperature

TABLE VI

Optimum Temperature for Milk Amylase Activity

INCUBATION TEMPERATURE	MILK MIXTURE ITSELF INCUBATED FOR 24 HOURS		LEAD SERUM INCUBATED 24 HOURS		IRON SERUM INCUBATED 24 HOURS	
	Increased reducing material	Iodine color	Increased reducing material	Iodine color	Increased reducing material	Iodine color
	*per cent**		*per cent**		*per cent**	
Blank (no starch)	**	—	**	—	**	—
30° C.	0.09	Yellow	0.15	Blue	0.07	Blue
35°	0.10	Yellow	0.17	Blue	0.07	Blue
40°	0.12	Yellow	0.17	Blue	0.10	Blue
50°	0.17	Red-blue	0.19	Blue	0.12	Blue
60°	0.12	Blue	0.17	Blue	0.10	Blue
70°	0.06	Blue	0.15	Blue	0.07	Blue
pH	6.71		5.88		3.48	

* Calculated as lactose hydrate.

** Blanks incubated at 30° and 70° for 24 hours showed no change in reducing power.

TABLE VII

Variation of amylase in individual cow's milk

COW NO.	NORMAL				COW NO.	MASTITIC			
	Reducing material		INCREASE	IODINE COLOR		Reducing material		INCREASE	IODINE COLOR
	Before incubation	After incubation				Before incubation	After incubation		
	*per cent**	*per cent**	*per cent**			*per cent**	*per cent**	*per cent**	
172	4.18	4.34	0.16	Red-blue	154	4.05	4.36	0.31	Red-blue
196	4.36	4.52	0.16	Red-blue	174	4.19	4.55	0.36	Yellow
177	4.61	4.77	0.16	Red-blue	180	3.93	4.17	0.24	Red-blue
252	4.40	4.58	0.18	Yellow	183	4.05	4.28	0.23	Yellow
253	4.81	4.94	0.13	Yellow	588	3.99	4.31	0.32	Yellow
530	4.28	4.46	0.18	Red	537 RF	2.67	2.96	0.29	Yellow
532	4.47	4.67	0.20	Red-blue	537 LF	2.74	3.26	0.52	Yellow
542	4.48	4.67	0.19	Yellow	537 RF	2.27	2.55	0.28	Yellow
526 RF**	4.12	4.27	0.15	Red	537 LH	3.76	4.07	0.31	Yellow
526 LF	4.46	4.62	0.16	Red	526 LH	2.10	2.48	0.38	Yellow
526 RH	4.34	4.58	0.24	Red					
	Mean		0.174			Mean		0.324	

* Calculated as lactose hydrate.

** Designation of quarters of udder:

RF = right front.
LF = left front.
RH = right rear.
LH = left rear.

is somewhere below 40° C. No detailed study was made to determine the reason for the lower degree of dextrinization in the case of the two sera, but the following differences in conditions are suggestive. The pH values of the reaction mixtures varied from 3.48 to 6.71 and the sera contained either iron or lead in solution. As shown in Table IV, when the starch is in sufficiently low concentration, color differences with iodine are sufficient to indicate dextrinization in the case of both lead and iron sera. Furthermore, it is quite possible that part of the dextrinizing factor may have been removed with the materials precipitated by the lead or iron. This was pointed out by Chrzaszez and Goralowna (5). The low pH value (3.48) of the iron-starch mixture may partially account for the decreased saccharifying activity.

Variation of amylase in milk. Colostrum and milk from diseased udders show greater dextrinizing activity than normal milk (Chrzaszez and Goralowna (5)), but nothing seems to have been reported concerning such milks in relation to the saccharifying enzyme. Evidence is fast accumulating to show that the incidence of chronic mastitis is becoming of increasing concern in market milk areas. The data shown in Table VII were accumulated primarily to yield more information on the variation in the saccharifying factor, but also to verify the variation in the dextrinizing factor in milks from normal and mastitic udders or quarters. For this purpose individual cows were selected from one herd; after discarding the first three streams, the samples were drawn into toluene, and the tests were made with a minimum of delay. Mixtures of 5 ml. milk and 1.5 ml. of 1.5 per cent starch were incubated for 24 hours at 37° C. Analyses for reducing material were made before and after incubation. It is evident that some variation exists within the milks of individual cows, whether normal or mastitic. Great differences are found in the milks from normal and mastitic animals, and some variation may be expected even between the milk from the quarters of the same cow. As a general rule it would seem that milk with a high saccharifying activity has high dextrinizing power.

DISCUSSION

The presence of a true diastase in milk is probably of greater theoretical interest than of practical importance either in the nutritional aspect or in connection with dairy technology. Our experiments have demonstrated that milk possesses raw starch liquefying power, soluble starch dextrinizing activity, and the ability to cause an increase in the reducing value of starch. Precautions have been taken to control the origin of the milk samples and to eliminate the possibility of the diastase being elaborated by bacteria as external contaminants of the milk. This was done by aseptic milking into toluene or by filtering the milk so drawn through sterile Pasteur-Chamberland filters. It was felt that these precautions were necessary before confirmation could be given to the report of Chrzaszez and Goralowna (5) in

the face of the findings of Kuttner and Somogyi (8) and others. This was especially true in view of the very limited and incomplete criteria given by the former authors.

If α-amylase is concerned with starch dextrinization and β-amylase is necessary to produce starch hydrolysis and yield products of higher reducing power than the original, then milk may be said to contain both α and β-amylase. The former is heat labile, its activity being greatly diminished at 55° C. for 30 minutes; the latter withstands heating at 65° C. for 30 minutes with no decrease in activity. These findings, when considered with the evidence of the great variability in the concentration of both the α and β forms in the milks of individual cows, enable one to understand why the starch-iodine reaction is not a safe criterion for the detection of improper low-temperature pasteurization of milk.

SUMMARY

Milk from normal cows has been shown to possess starch liquefying, starch dextrinizing and starch saccharifying activity. Although it did not seem feasible to make optical rotation studies, because of the high lactose concentration and rather weak diastatic activity of milk, it appears justifiable to assume that milk contains an α-amylase as well as a β-amylase. The former is less heat stable than the latter, being considerably inactivated by heating at 55° C. for 30 minutes. The β-amylase of milk maintains its original activity after a period of 30 minutes at 65° C.

The optimum temperature of incubation for determining the α-amylase potency of milk is in the zone 30–40° C., that for the β type is approximately 50° C.

Milk from cows showing evidences of chronic mastitis is characterized by a high, though variable, diastatic activity.

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American Dairy Science Association Announcements

STUDENTS' NATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

The team from Ohio State University won highest honors in the dairy products judging contest held in Atlantic City, New Jersey, on October 12, in connection with the Dairy Industries Exposition. The large silver cup which is awarded the team winning first place in judging all products becomes the permanent property of Ohio State University, teams from that institution having won it three times—1933, 1934, and 1936.

Connecticut State College was a close second, followed by Cornell University, University of Nebraska, University of Minnesota, and University of Tennessee. Team from 18 agricultural colleges competed.

These six colleges were each awarded a scholarship to be assigned to any member of the team (including the alternate) whom the college may select. To be eligible for a scholarship, the team member must be a senior and his undergraduate work must be of such character and content as to qualify him to take graduate work leading to a Master's degree in dairy industry.

The six scholarships are annually provided by The Dairy and Ice Cream Machinery and Supplies Association, Inc., which association has for seven years joined with the American Dairy Science Association in sponsoring this contest. The former association also provides five silver cups which are awarded to the team scoring first place in judging all products and the teams winning first place in the judging of butter, cheese, milk, and ice cream; also gold, silver and bronze medals to the individual contestants who win first, second, and third places, respectively, in judging each product and all products. The winning contestant in judging milk was also awarded a gold medal by The International Association of Milk Dealers, and the winner in judging ice cream a gold medal by the International Association of Ice Cream Manufacturers.

Following are the teams and individuals who won high standings in the contest:

ALL PRODUCTS

Teams		*Individuals*	
1—Ohio State University	308.60	1—Joseph Adams, Ohio State University	93.75
2—Connecticut State College	315.85	2—Albert S. Tomlinson, Cornell University	94.00
3—Cornell University	342.35	3—Oscar H. Johnson, Connecticut State College	98.05
4—University of Nebraska	373.35	4—Gifford G. Danke, University of Wisconsin	99.85
5—University of Minnesota	374.00	5—John F. Rowlson, Connecticut State College	102.25
6—University of Tennessee	376.70		

BUTTER

Teams		*Individuals*	
1—Iowa State College	60.75	1—John T. Griffith, Iowa State College	12.50
2—University of Minnesota	65.00	2—Henry E. Butler, University of Maryland	12.75
3—Michigan State College	66.50	3—John C. Pfeffer, University of Illinois	14.50
4—Connecticut State College	70.50	4—R. D. Kilpatrick, University of Tennessee	15.50
5—University of Wisconsin	72.25	5—Gifford G. Danke, University of Wisconsin	16.50

ICE CREAM

Teams		*Individuals*	
1—Cornell University	82.00	1—Albert S. Tomlinson, Cornell University	19.50
2—Connecticut State College	86.10	2—Gifford G. Danke, University of Wisconsin	24.10
3—Purdue University	86.90	3—F. W. Skolton, Ontario Agricultural College	24.60
4—University of Minnesota	89.80	4—Oscar H. Johnson, Connecticut State College	24.80
5—Ohio State University	89.90	5—Joseph Adams, Ohio State University	25.00

MILK

Teams		*Individuals*	
1—Ohio State University	68.45	1—Russell Fifer, Ohio State University	19.70
2—Pennsylvania State College	83.80	2—Albert S. Tomlinson, Cornell University	21.00
3—Connecticut State College	86.75	3—James B. Hamilton, Ohio State University	22.25
4—Cornell University	89.10	4—Gifford G. Danke, University of Wisconsin	23.25
5—University of Nebraska	92.50	5—Herman Openlander, Michigan State College	24.25
		5—John F. Rowlson, Connecticut State College	24.25

CHEESE

Teams		*Individuals*	
1—University of Tennessee	66.00	1—William M. Roberts, University of Tennessee	17.00
2—Connecticut State College	72.50	2—Maynard C. Stearns, University of Wisconsin	19.00
3—Cornell University	75.50	3—Grant Hartman, University of Illinois	21.00
4—Ohio State University	76.25	4—F. G. Warron, Kansas State College	21.25
5—University of Nebraska	82.00	5—John B. Wilcox, Cornell University	22.25

ERRATA

Page 171. The second sentence of the second paragraph should read as follows: "To meet the legal standard and to provide a factor of safety, it became the occasional practice of some milk distributors to standardize solids-not-fat by the addition of condensed skimmilk, the amount used varying with the season of the year."

Page 338. Insert at bottom of formula for lactose desoxycholate agar the following: "neutral red ..... 0.33 grams."

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ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

Published in cooperation with
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United States Department of Agriculture

ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

BUTTER

Creamery Business Analysis. L. L. Ullyot and H. F. Hollands, Div. of Agr. Ec., Univ. of Minn., St. Paul, Minn. Minn. Agr. Exp. Sta. Bul. 322, April, 1935.

The authors present an analysis of the business of 117 Minnesota cooperative creameries. The purposes of the bulletin are to indicate the general types of information that should be included in an annual creamery report, to show patrons and other persons how to analyze and interpret such a report, and to indicate the general financial condition and the operating efficiency of the cooperative creameries included in the study.

An annual report should provide the specific information needed to determine, first, the financial status of a business, and, second, the results of the year's business operations. To serve these two purposes adequately, the report must contain the following three statements:

1. A balance sheet, which is a statement of the financial conditions of a business at the time the annual report is made.

2. An operating statement, which shows the various sources of income and the kinds of expenses incurred.

3. A statistical statement, which shows the quantities of commodities handled and the average prices received and paid for these commodities. From these data may be determined such information as the per cent of overrun and the expenses per pound of butter made. S.T.C.

CONCENTRATED AND DRY MILK

A Method to Obtain the Desired Fat Content of Dry Milk. J. Brabec. Mlekarske Listy 27, 231–2 (1935); Chem. Obzor II, Abstract sect. 74.

The desired fat content of dry milk can be calculated according to the formula $t = \frac{T}{95 - T}\left(0.09 + \frac{t' + L}{4}\right)$ where T is percentage of desired fat content, t' the fat content of milk used, t the fat content to which the milk must be standardized before drying and L is the value found by the lactometer. J.J.K.

Dry Skimmilk—How to Use It. Alice M. Child, Div. of Home Ec., Univ. of Minn., St. Paul, Minn. Minn. Agr. Exp. Sta. Bul. 313, Dec., 1934.

In homes where the amount of milk is limited by the cost, the necessity for economy often results in an insufficient milk supply. By the use of dry

skimmilk in cooking and whole milk for drinking, it is possible to bring the total milk consumption to the desired amount. When dry skimmilk is used in place of part of the necessary milk supply, certain food materials must be added to the list to take the place of butterfat in building up a strong resistance to disease.

Directions and tested recipes for the use of dry skimmilk in home and quantity cookery are given. S.T.C.

CHEESE

A Study of the Bacterial Flora of Foremilk and of Rennet Extract, With Special Reference to Acid-proteolytic Types. M. R. KNOWLES, Agri. Bact. Dept., Univ. of Reading. J. Dairy Research 7, 1, p. 63, Jan., 1936.

A relatively small number of acid-proteolytic cocci was found in the foremilk obtained aseptically from our healthy cows. Of 58 cultures of cocci isolated only four types were capable of digesting casein under acid conditions brought about by their own activities, namely, Staphylococcus sp., S. liquefaciens, Micrococcus caseolyticus, M. freudenreichii. Only the first two were vigorously acid-proteolytic. Gelatin was liquefied by 87.3 per cent of these cultures and 23.7 per cent were acid-proteolytic in milk. Of the gelatin liquefiers only 27.1 per cent liquefied milk. The remaining strains, however, may aid in the final breakdown of split-protein products in ripening cheese.

Rennet extract contained a varied flora of proteolytic cocci that under normal conditions would gain entrance into the cheese. The types found in fresh extracts were similar to those found in the foremilk and it is considered likely that the organisms in the rennet extract are the same organisms transplanted from the udder of the cow to the stomach of the suckling calf and thence to the rennet extract. In the samples of old rennet many contaminating types of organisms were found, including yeasts, molds and actinomyces. H.A.B.

The Varieties of Cheese in the International Market. JAR. DVORAK. Mlekarske Listy, 26, 12, 29–31 (1934); Chem. Obzor II, Abstract sect. 73.

A review of the original varieties of cheeses manufactured in various countries, with data on milk and fat composition elaborated by the International Cheese Commission at the federation in Brussels. J.J.K.

Lattopect. L. HERTL. Mlekarske Listy 27, 210–2 (1935); Chem. Obzor II, Abstract sect. 74.

Lattopect, a fruit pectin used in milk products, when used in small amounts (2 per cent) improves the cheese and prevents drying out. J.J.K.

(Conservatives) Preservatives for Melted Cheese. K. KOUTNY. Mlefiarske Listy 27, 6–10 (1935); Chem. Obzor II, Abstract sect. 74.

The preservatives (benzoic acid, sodium benzoate, etc.) should not be used for melted cheese. J.J.K.

ICE CREAM

Drum vs. Spray Process Dry Milk in Ice Cream. R. L. CARITHERS AND W. B. COMBS, Univ. of Minn., St. Paul, Minn. Ice Cream Rev. *19*, 8, p. 42, March, 1936.

Dry milks were prepared by the spray process and the atmospheric drum process for use in ice cream. Preheating of skimmilk manufactured into drum process dry milk to temperatures of 63° F., 145° F., and 185° F., had no effect upon the quality of ice cream made using these dry milks. It was not possible to detect any difference in the quality of ice cream made using fresh dry skimmilks prepared by the spray process and the drum process as compared with ice cream made using plain condensed milk. After storing the dry milks for six months, however, the flavor of those made by the drum process dried milk carried over into ice cream to such an extent that it had a decided detrimental effect on the flavor. J.H.E.

Causes of Sandiness in Ice Cream in the Winter Months. H. H. SOMMER, Univ. of Wis., Madison, Wis. Ice Cream Rev. *19*, 8, p. 44, March, 1936.

Slower turnover of ice cream in winter is not the only reason for the greater likelihood of sandiness in winter. If no change is made in the mix formula as the colder months of the year approach, the serum solids content of the mix will actually be higher than during the summer months, due to the fact that the solids-not-fat content of milk is higher in winter months. A formula for arriving at the serum solids content that may be safely used is given. J.H.E.

Effect of Freezing and Homogenization on the Colloidal Stability of Mixes. H. H. SOMMER, Univ. of Wis., Madison, Wis. Ice Cream Rev. *19*, p. 3, p. 56, Oct., 1935.

The colloidal stability of ice cream mixes is becoming increasingly more important with the introduction of two-stage homogenization and continuous freezers. Homogenization and freezing are recognized as destabilizing factors in the case of emulsions and colloidal sols. If the mix is not sufficiently stable the ice cream on melting is likely to present a curdy and wheyed-off appearance. This defect is associated with the ability of proteins to retain water. The author reviews the work of Hans Mumm which indicates that hydration of casein increases on either side of the isoelectric point. This

would mean that the ability of milk proteins to retain water decreases as the acidity increases. The finding that previous high temperature treatment increases the hydration of casein suggests that mix pasteurizing temperatures have an effect on the ability of the milk proteins to retain water in ice cream. J.H.E.

Ice Cream Mix As a Solution. H. H. SOMMER, Univ. of Wis., Madison, Wis. Ice Cream Rev. *19,* p. 46, Dec., 1935; *ibid., 19,* 6, p. 62, Jan., 1936; *ibid., 19,* 7, p. 66, Feb., 1936.

The author states that a full understanding of whipping ability, mix viscosity, fat clumping, shrinkage of ice cream, crumbly body, sticky body and serum drainage or curdled appearance of the melted ice cream require the proper conception of the mix as a solution. In these three articles the mix constituents are classified and discussed according to their state of suspension. It is pointed out that the milk proteins and stabilizers exist in the mix in the form of colloidal suspensions and the fat exists in the form of an emulsion. In the case of the fat emulsion the size of the globules, the nature of the adsorption film which surrounds them, and the electric charge carried by them determine the behavior of the emulsion. In the case of the colloidal protein suspensions, the colloidal particle size, their hydration and the electric charge carried by them determine their stability.

The electric charge on the suspended particles in both cases play a very important part in determining their physical behavior. Factors discussed as affecting the charge on the colloidal protein particles in milk and in ice cream mix are as follows: (1) acidity, (2) salts (ions), (3) temperature, (4) previous heat treatment, (5) rennin action.

The observed destabilization of proteins by freezing may be due to increased acidity, increased salt concentration, a changed ion concentration with the positively charged ions more predominant, a shift in the isoelectric point of the proteins, and/or decreased hydration of the proteins. Destabilization occurs only insofar as aggregation of the particles occurs. As freezing progresses, decreased kinetic energy and increased viscosity make aggregation more difficult. On this account quick freezing has less destabilizing effect than slow freezing for in slow freezing the product lingers longer in the zone of maximum unstability. J.H.E.

Sherbets—How to Improve Them. W. C. COMBS, Univ. of Minn., St. Paul, Minn. Ice Cream Rev. *19,* 6, p. 38, Jan., 1936.

The author reviews the observations that were made on 19 sherbets exhibited from various parts of Minnesota and the Northwest. Considerable variation in total solids and acidity was observed. The total solids content averaged 32.9 per cent. The range was from 26.8 to 39.4 per cent. The acidity of the sherbets, expressed as citric acid, ranged from 0.27 to 0.75 per

cent with an average of 0.43 per cent. The sherbets graded good or very good, had an acidity of not more than (or slightly above) 0.45 per cent and a total solids content of not below 30 per cent.

Careful standardization of composition and acidity are recommended. Sherbets should not be whipped in the freezer to contain more than 40 per cent swell. Best results will be secured by reducing the amount of sherbet in the freezer and adopting methods of freezing inducive to quick freezing. A small amount, three per cent, of sodium caseinate added to sherbet has been observed to bring about marked improvement in body and texture. (The manufacture of frozen deserts with sodium caseinate is patented.)

J.H.E.

This Special Mold Business. (No author given). Ice Cream Rev. *19*, 5, p. 30, Dec., 1935.

The history and development of the ice cream mold is given as well as the procedure for designing and manufacturing metal molds. J.H.E.

Recent Legislative Trends as They Affect the Ice Cream Industry. Edward Thom, Assoc. Editor, Ice Cream Rev., Milwaukee, Wis. Ice Cream Rev. *19*, 9, p. 40, April, 1936.

Recent state laws dealing with pasteurization of ice cream mix and overrun control of ice cream are reviewed. There is growing sentiment among health officials that ice cream, susceptible to contamination much as milk is, should be handled as milk is. J.H.E.

Operating the Refrigeration System Economically. F. B. Gilbert, Res. Engineer, The Creamery Package Manufacturing Co., Chicago, Ill. The Dairy World. *15*, 2, p. 41, July, 1936.

The author discusses ways and means of checking the efficiency of operation of a refrigeration system, pointing out the symptoms and causes of some of the common maladjustments encountered in commercial dairy plants. By comparing temperatures and pressures on the suction and discharge lines with theoretical tables or charts indications of inefficient operation are obtained and the causes can be ascertained by simple elimination. Generally speaking any change in the operation of the system which causes head pressures to rise or suction pressures to decrease will result in more power cost per ton of refrigeration. Proper maintenance of condensers, evaporators and storage rooms is also briefly considered as well as the amount and purity of the ammonia in the system. M.J.D.

Heat Insulation. Harvey B. Londsay, Dry Zero Corp., Ice and Refrig. *90*, 3, p. 194, March, 1936.

Conduction is the major means of heat travel through cellular and interstitial materials. The transfer of heat in a gas filtered space is a function

of the molecules of gas in the space. The nature of their action is the same whether a small number is confined in a small space or a large number confined in a larger space. Heat affects the movements of these molecules. The higher the heat load the greater is this heat agitation. The flying molecules rebound very rapidly from a smooth hard surface, and are gone before they can accumulate a heat load. In the case of a soft surface they are momentarily imbedded while in the case of a rough surface they may ricochet from surface to surface. Thus they pick up a greater heat load and surfaces of the latter two types tend to increase the rate of heat transfer.

Moisture in the insulating material greatly affects its efficiency. Thus far no effective method of keeping moisture completely out of insulation has been developed. Data are included to show how household refrigerators insulated with a non-hygroscopic insulation lost but 7 per cent in efficiency in the same period. L.C.T.

MILK

Effect of a Milk Plant Quality Program on the Price Paid to the Producers for Milk. V. C. MANHART, Dept. of Dairy Husb., Purdue Univ., Agr. Exp. Sta., Lafayette, Ind. Purdue Exp. Sta. Bull. No. 404, 1936.

A four year study of a milk quality program as used by an Indiana milk plant was made to determine the equitableness of the program to producers. The program was based on a grading system in which the grades of milk were determined by flavor, the methylene blue test, and the sediment test.

The results show that the program was "undoubtedly equitable to all producers and decidedly advantageous to a large majority of the producers." Most of the producers secured larger returns under the program than they would had they sold their product at the prevailing market price without regard to grade. B.E.H.

Milk Quality Improvement Effected at the Farm by a Plant Program. V. C. MANHART AND A. V. MOORE, Dept. of Dairy Husb., Purdue Univ., Agr. Exp. Sta., Lafayette, Indiana. Purdue Exp. Sta. Bull. No. 405, 1936.

This bulletin describes a simple, inexpensive milk quality program as put into effect by an Indiana dairy plant. Three grades were established; premium, regular and low. The milk was first graded at the plant on flavor and the objectionable flavored milk was either rejected or placed in a low grade without further testing. Satisfactory flavored milk was subjected to the methylene blue and sediment tests and the grade was determined by these tests.

The authors summarize the benefits derived from the program as follows:

"The milk plant received a better quality milk which when sold as fluid milk commanded a price of more than one cent a quart higher than the prevailing price for pasteurized milk in the same market.

"The consumer received a good quality milk, wholesome in nature, pleasing in flavor, and with a total bacterial count of less than 10,000 per cc. after pasteurization and at the time of delivery.

"The producer of premium milk received a premium of about 4.5 cents per pound milk fat which represented an increase of more than 17 per cent over the prevailing price paid in the territory. Furthermore this benefit was received by a majority of the producers.

"In view of the foregoing considerations, the quality program reported herein is recommended to milk plants as highly satisfactory for the improvement and maintenance of quality in the raw milk supply." B.E.H.

A National Yardstick of Milk Quality Factors. RUSSELL J. DAVIS. Milk Dealer *25*, 11, p. 36, August, 1936.

The author discusses the present lack of knowledge of the difference between the various grades of milk. He contends that due to the present haphazard understanding of milk quality, a national yardstick, a "common denominator" of quality factors, is needed if a uniform understanding of milk quality is to be gained and sales of the better milks of all grades are to be increased. A yardstick of quality factors is presented. C.J.B.

Milk Consumption Increasing in New York Metropolitan Area. Milk Dealer *25*, 11, p. 86, August, 1936.

A report of milk consumption in the New York metropolitan area is shown by a study made by the Milk Research Council. C.J.B.

The Determination of Lactose and Glucose in Milk. TUDOR STANLEY AND GEORGE JONES, Nat. Inst. for Res. in Dairying, Univ. of Reading. J. Dairy Research *7*, 1, p. 41, Jan., 1936.

The presence of glucose in milk has been difficult to determine due to methods of analysis. In this experiment glucose was determined in milk with remarkable accuracy as the reducing material removable by washed yeast at a neutral or slightly acid reaction. The washed yeast was found not to ferment lactose and did not add reducing material to tungstic filtrates.

Typical results obtained by this method was as follows:

Total reducing sugar 4.52 per cent, lactose 4.36 per cent, glucose 0.08 per cent for a normal cow.

Total reducing sugar 4.20 per cent, lactose 3.98 per cent, glucose 0.11 per cent for a cow late in lactation. H.A.B.

Determination of the Quality of Milk from Its Specific Gravity and Fat Content with Lactonogram. Bycichin. Sbornik Ceskoslov. Akad. Zemedelske II, 220–7 (227 in French and Russian), 1936.

Various methods of determination of quality of milk and its composition are reviewed and the use of specially composed lactonograms (a graph from which total solids may be read if the fat content and lactometer readings are known) is suggested giving total dry matter, dry matter free of fat, fat content in dry matter, and of added water and skimmilk from the specific gravity and fat content of investigated milk. J.J.K.

Determination of Freezing Point of Milk for Testing the Presence of Water in Diluted Milk. Mlekarske Litsy 26, 71–3 (1934); Chem. Obzor II, Abstract sect. 73.

The freezing point was found to have a value between $-0.59°$ and $0.50°$; the average $= -0.5552°$ but samples of milk showed variable degrees of supercooling in making the determination. No relation between freezing point and season was found in the months March–June; the freezing of milk was slower in the hot season than in the cold season. J.J.K.

Test for Pasteurized Milk. K. Koutny. Mlekarske Listy 26, 246–9 (1934); Chem. Obzor II, Abstract sect. 73.

A review of bacteriological and chemico-biological changes of milk in pasteurizing milk to 60–63° C. and above 80° C. The milk is tested for short time pasteurization (above 85° for at least 1 minute) by 3 per cent alcohol solution in guaiacol and 1 per cent solution of hydrogen peroxide. An analogous test for long time pasteurization is not known. J.J.K.

Biennial Reviews of the Progress of Dairy Science. Section C, Dairy Chemistry. W. L. Davies, Nat. Inst. for Res. in Dairying, Univ. of Reading. J. Dairy Research *7*, 1, p. 75, Jan., 1936.

The review records the progress of dairy chemistry for the period extending from the end of that dealt with in a previous review (1933) to August, 1935. The subjects noted as arousing the greatest interest during the last two years are: composition and autoxidation of butterfat, the composition of casein, the physical chemistry of milk and its products and the technology of butter and cheese manufacture.

The review covers the composition of milk, chemistry of milk constituents, physical chemistry of milk and certain factors concerned with butter, cheese, concentrated and dried milk products. H.A.B.

The Effect of Variations in Feeding on Dairy Cows Yielding Milk of Poor Quality. Alice Watson Stewart and James Fowler Tocher, the Rowett Res. Inst. and the Univ. of Aberdeen. J. Dairy Research *7*, 1, p. 1, Jan., 1936.

To find a practicable means of improving the quantity and quality of the milk produced by cows consistently yielding milk of a solid-not-fat content

studied during three lactations using two Friesian cows, one Guernsey and one Shorthorn Cross cow.

1. Good grazing with a supplementary ration
2. Well balanced normal rations
3. High protein rations
4. High carbohydrate rations

It was concluded that the solids-not-fat content as well as the quantity of milk produced may be slightly raised by improved feeding practices, but that a high solids-not-fat content is principally a hereditary factor. H.A.B.

The Metabolism of Betaine and Allied Tertiary Nitrogenous Bases in the Ruminant. WILLIAM LEWIS DAVIES, Nat. Inst. for Res. in Dairying, Univ. of Reading. J. Dairy Research 7, 1, p. 14, Jan., 1936.

The origin of and means of preventing fishy flavors in the milk of cows fed on sugar-beet by-products was investigated. Beet molasses contains on the average 1.5 per cent betaine nitrogen, molasses beet pulp 0.5 per cent, and fresh beet tops 0.03 per cent, so that dairy cows on normal rations containing suitable quantities of these products may be receiving up to 100 grams of betaine per day. These feeds also contain traces of the tertiary nitrogenous bases, choline, trimethylamine and trimethylamine oxide. The tertiary bases, especially betaine, are regarded as the precursors of the fishy taint of milk. The main tertiary metabolite of betaine was found to be trimethylamine oxide, which is more chemically active than betaine itself. With an intake of 100 grams betaine, the peak of trimethylamine oxide excretion in the urine was found to occur in 4½ hours. The maximum concentration of the oxide in the blood probably occurs shortly before the peak of excretion in the urine is reached. Consequently, beet products should be fed as far away from the subsequent milking time as possible, preferably after milking. The mechanism of the development of the fishy taint in milk by interaction of the precursor with one or more of the milk constituents, is being investigated and the work will be reported in a later paper.

H.A.B.

The Chlorine Content of Milk as an Indication of Mastitis. J. W. BLOOD AND A. ROWLANDS, Midland Agri. Coll., Sutton Bonington, Loughborough. J. Dairy Research 7, 1, p. 47, Jan., 1936.

The authors studied the reliability of various methods of chlorine determination as means of detecting mastitis in cows. They found that the direct titration of chlorine in milk with silver nitrate solution, without the removal of proteins, always yielded high results. With 10 ml. milk, 40 ml. distilled water and 10 drops of potassium chromate used in the titration with 0.1 N silver nitrate, the results were 25 per cent high and the endpoint was poor. With 10 ml. of milk, 80 ml. distilled water and 2 ml. potassium chromate, the results were still 15 per cent high, although the endpoint was improved.

Three other methods in which the protein was removed or destroyed first yielded comparable and reliable results. However, the arbitrary standard of 0.14 per cent chlorine fixed by Rosell as the maximum content of normal milk was not found justifiable, because cultural examinations often revealed no evidence of mastitis when a chlorine content above this limit was found and infected udders at times yielded milk with a chlorine content within the range of normal milk, *i.e.*, 0.07–0.12 per cent. H.A.B.

Note on the Sulphydril Compounds of Milk. CHRISTOPHER JAMES JACKSON, Dept. of Biochem., Univ. of Alberta, Edmonton, Canada. J. Dairy Research *7*, 1, p. 29, Jan., 1936.

Experiments with the nitroprusside test on a large number of milks seem to indicate that there are no sulphydryl compounds in free solution in cow's milk. The occurrence of a positive nitroprusside test in milk treated with sodium cyanide is considered to be due to the cystine in the protein complex. H.A.B.

Improving Milk Products by Yeast Cultures. O. LAXA. Mlekarske Listy 26, 339–40 (1934); Chem. Obzor II, Abstract sect. 73–4.

Yeast cultures decomposing milk sugar with the formation of perfume esters improve also the taste of milk. J.J.K.

Correct Evaluation of the Agglutination Test with Bang Antigen. KARL DIERNHOFER. Z. Infektion. Parasitäre Krankheiten Hygiene Haustiere *49*, 2, p. 146, 1936.

This is a discussion of the methods used in evaluating the readings obtained in testing for abortion disease. L.D.B.

Results of Five Years' Study of Mastitis with Special Emphasis on the Effect of an Artificial Dry Period. OTTO ROEMMELE. Z. Infektion. Parasitäre Krankheiten Hygiene Haustiere *49*, 2, p. 123, 1936.

From previous investigations of the numerous methods for the certain and rapid identification of mastitis this writer considers none of them is entirely satisfactory. Beeker had found that the laboratory tests were superior to those recommended for field use, but that continued observations of the condition of the udder and characters of the milk by the milker were a more certain means of diagnosing the disease than the tests usually recommended. He suggests that an expert should be able to determine these abnormal conditions according to flavor, odor, and appearance on the same basis on which other products are judged.

The treatment of 826 animals with Entozon once or twice exerted little influence on the course of the disease. However, treatment shortly before the animal ceases to give milk or during the dry period was most suitable for curing the condition. The use of infusions of Entozon, 1 to 500 or 1 to

800, can be used to hasten a cure during the dry period. However, the treatment may be given at any time, but sometimes acts in an unfavorable manner because of the reduced milk flow after the cow freshens again. These recoveries were seldom permanent and may have started because of reinfection.

L.D.B.

Studies on the Infection of the Udder by the Abortion Bacillus of Bang, and Methods for Its Detection. W. STOCKMAYER. Z. Infektion. Parasitare Krankheiten Hygiene Haustiere *49*, 1, p. 46, 1936.

The bacteriological examinations started at the time of parturition, using a gentian-violet-malachite green agar, liver agar and other special media, and followed through the lactation period. The experimental animals consisted of 39 cows, previously found to be infected in the udder. The agglutination titers of the blood sera of these cows varied between 1000 and 50,000.

As determined by plate count, the elimination of the abortion bacillus from the infected animals was greatest the first day of lactation, and decreased rapidly during the next two weeks. However, this udder infection may persist throughout the lactation period, or may cease suddenly. In one animal the infection did not start until 10 days after the first calving, but persisted through the second lactation period. The number in some animals reached to 10,000 to 200,000 organisms per cc., but in most instances it was below 2,000 per cc. No doubt there were many more organisms than could be determined by the plate method in all cases. At certain times there was no clinical evidence of an udder infection, and in some animals positive evidence was found only on continued search.

Permanent carriers possessed high agglutination titers of the blood and milk serum. (In the preparation of the milk serum the fat was removed by chloroform). In some animals with negative or low titer agglutinins in the milk serum the organisms were occasionally present. It was evident that the organism might set up an independent infection of the udder. Guinea pig inoculation was the most reliable means of diagnosing this condition, although culturing on the gentian-violet-malachite green agar plates was very satisfactory. Cultural methods were more satisfactory in case the guinea pigs had already become infected from other sources. The use of animals was found to be time consuming (6–8 weeks) and expensive. However, the culture methods used were not widely enough known to be of much importance in detecting such animals. Also the serological reactions were not above doubt since some carriers (chiefly intermittent) might not contain agglutinins in the blood serum or milk serum. L.D.B.

Can Udder Tuberculosis be Diagnosed by Means of a Cell Count of the Milk? C. EHRLICH. Z. Infektion. Parasitäre Krankheiten Hygiene Haustiere *49*, 2, p. 97, 1936.

The author concluded that: The cell content of milk is much more difficult to determine than that of blood. Many of the cells in milk have disintegrated so that they do not stain readily and are difficult to classify. It is especially difficult to distinguish the epithelial cells and monocytes.

The following classification is offered to differentiate between the cell counts observed in tuberculosis of the udder and mastitis: From tuberculous udder—6.5 per cent lymphocytes 35.0 per cent polymorphonuclear leucocytes, 55.0 per cent monocytes, 3.5 per cent epithelial cells. From a mastitis udder—6.5 per cent lymphocytes, 68.8 per cent polymorphonuclear leucocytes, 22.4 per cent monocytes, 2.3 per cent epithelial cells.

The difference between the appearance of the two preparations is not so evident that they can be used for purposes of differential diagnosis without an analysis of this sort. It is not possible to utilize the same preparations, both for demonstrating tubercle bacilli and cell counting, because of the difficulty in recognizing epithelial and giant cells with high magnifications. The presence of giant cells and groups of epithelial cells are considered to be pathognomonic of udder tuberculosis. This writer found them in 49.2 per cent of the animals investigated. No giant cells of epitheloid cell groups are found in cases of mastitis. L.D.B.

Detection of Shedders of Streptococci Responsible for Infectious Bovine Mastitis. W. N. Plastridge and E. O. Anderson, Storrs Agr. Exp. Sta., Storrs, Conn. Am. J. Pub. Health *26*, 7, p. 711, July, 1936.

The term "shedders" is used to designate lactating animals which eliminate the organism in question through the milk. The following determinations were made on 970 quarter samples from 15 herds: physical appearance, brom thymol blue reaction, leucocyte count, sediment volume, types of colonies on blood agar plates and microscopic examination of incubated samples.

A plan is described for differentiating *Streptococcus mastitidis* (Group A), the common cause of infectious chronic mastitis, from weakly pathogenic (Group B) and saprophytic streptococci of bovine origin.

Microscopic examination of incubated milk samples was found to be the most effective of six methods used in detecting shedders of *Streptococcus mastitidis*. This method revealed the presence of streptococci in 98.7 per cent of the samples collected from animals affected with chronic streptococci mastitis. However, about 13.5 per cent of the samples from healthy quarters also yielded chains of streptococci by this method. It is concluded that the significance of the finding of streptococci in incubated samples from healthy quarters, in the absence of other evidence of mastitis, or during the first 2 and last 4 weeks of the lactation period, can be determined only by isolation and identification of the streptococcus found. M.W.Y.

Staphylococcus Food Poisoning. Report of a Small Milk-borne Epidemic. H. J. SHAUGHNESSY AND T. C. GRUBB, Ill. State Dept. of Pub. Health. J. Inf. Diseases *58*, 3, p. 318, May–June, 1936.

On the basis of circumstantial evidence, an epidemic of food poisoning involving 25 persons was ascribed to the consumption of raw milk from cows with a staphylococcus mastitis and of ice cream made from such milk. One strain of hemolytic staphylococcus isolated from the milk was shown to produce, under suitable laboratory cultivation, a toxic substance capable of causing vomiting in monkeys and man. Negative results were obtained from feeding experiments with other strains of staphylococci from the milk, but these results were thought to be due to the insusceptibility of some of the monkeys to the enterotoxin. W.C.F.

A Differential Study of Forty Brucella Strains Isolated in Minnesota. P. KABLER AND M. MACLANAHAN, Dept. of Pub. Health. J. Inf. Diseases *58*, 3, p. 293, May–June, 1936.

The 40 strains of *Brucella* were classified as follows: 25 of *Br. suis*, 13 of *Br. abortus* and 2 with conflicting reactions, one apparently *Br. suis* and the other *Br. melitensis*. The results indicated that in Minnesota *Br. suis* is the etiological agent of human cases of undulant fever about twice as often as *Br. abortus*. The original oxygen tension requirement together with the dye plate growth characteristics gave a fairly reliable means of differentiating the *Brucella* strains, while the agglutinin absorption method was of little or no value. The dyes used in the plates were basic fuchsin, thionin and pyronin. W.C.F.

Why Frequent Health Examinations of Employees Are Needed. F. W. FABIAN, Dept. of Bact. and Hygiene, Mich. State Coll., East Lansing, Mich. Ice Cream Field *28*, 2, p. 9, June, 1936.

The author summarizes his recommendations regarding the health examination of employees in the food and dairy industries as follows:

1. That every employee should be required to take a complete medical examination by a competent physician and submit the necessary samples of blood, feces and urine for laboratory examinations, together with any other tests necessary, at the time he is employed and semi-annually thereafter. If the board of health doesn't require such an examination, the employer should, for his own protection.

2. That supplementing the above examination and as an added protection, all other employees coming in direct contact with food, milk or other dairy products should be examined daily by a nurse, foreman or some other person for evidences of contagious diseases. By means of education and intelligent application, many cases of potential or actual disease may be found in this way.

3. Whether this system or any other is used in trying to find persons suffering with contagious disease, they must not be penalized by being discharged or temporarily laid off without due compensation.

4. Only persons who are inherently clean should be employed in the food or dairy industry. All new employees should be watched carefully until they have been so classified. W.C.C.

Investigation Shows Bacteria in Air May Survive for Days. Milk Dealer *25*, 11, p. 47, August, 1936.

A short description of an investigation to determine how long bacteria live suspended in air. The most important conclusion made is that respiratory organisms are apparently "tough" enough to adapt themselves for air transmission, while those of the intestinal tract depend for transmission almost entirely on food and water. C.J.B.

Technique for Obtaining Anaerobic Milk, With Some Observations on Its CO_2 Content. CHRISTOPHER JAMES JACKSON, Dept. of Bioch., Univ. of Alberta, Edmonton, Alberta. J. Dairy Research *7*, 1, p. 25, Jan., 1936.

A simple method is described for obtaining milk anaerobically from the udder, for transferring it to an electrode vessel for the determination of oxidation-reduction potentials, and for transferring it to a Van Slyke apparatus for gas analysis. The total amount of CO_2 in cows' milk was found to be about half that of their blood plasma varying from 53.8 vol. per cent to 59.8 vol. per cent in the blood plasma and from 25.8 vol. per cent to 28.7 per cent in the milk of six cows. H.A.B.

Bacteriophage Phenomena in Cultures of Lactic Streptococci. H. R. WHITEHEAD AND G. A. COX, Dairy Res. Inst. (N. Z.), Dept. of Sci. and Ind. Res., Palmerton, North New Zealand. J. Dairy Research *7*, 1, p. 55, Jan., 1936.

Bacteriophages were isolated from cultures of lactic streptococci ("starter cultures") and their properties and modes of action described. The presence of phage in a starter culture was first recognized during a renewed investigation of the phenomena of failure in activity caused by aeration of the milk at the time of inoculation. The mother culture would appear normal. A batch of starter prepared from the mother culture in vigorously stirred milk was normal in appearance and acidity, but it failed to produce acid when added to milk in the cheese vat. The same starters in unstirred milk produced acid normally. When a few drops of the active and of the inactive starter were added to tubes of pasteurized milk colored with methylene blue and held at 30° C., both tubes decolorized in 2–3 hours and smears showed copious growth of normal streptococci in both tubes. An hour later, however, the blue color had returned to the tube containing the inactive starter and a smear showed the absence of bacteria. The active starter grew normally and clotted the milk. The lytic agent or phage could be transmitted to

tubes of active young cultures of the susceptible strain by transferring minute quantities of the milk in which the streptococci had undergone lysis.

When a clotted starter culture which was known to be inactive was spread thickly on the surface of yeast whey sugar and incubated overnight at 30° C. the dense, almost confluent growth was dotted with clear circular "plagues." When the same inactive culture was streaked sparingly over the surface of the agar to produce discrete colonies at 30° C., a proportion of the colonies gradually became transparent until only their "ghosts" remained. The phage could be propagated from these transparent colonies.

Purification of the phage was accomplished by adding rennet and calcium chloride to sterile milk which had been inoculated with the susceptible streptococcus and had been phaged in four to five hours by a drop of phaged culture. The whey from the resulting clot was filtered through linen and a sterilized Seitz filter yielding a clear fluid free from bacteria and containing a high concentration of phage, which was kept at 4° C. for several months without significant loss of strength. It was almost completely destroyed by exposure to 70° C. for 30 minutes.

The most striking feature of this phage was its great activity, various preparations giving plaque counts of hundreds of thousands of millions per ml. Its optimum temperature was about 30° C., acting more slowly at 20 and 37° C., so that it might be only slightly active in the starter, but quite active in the cheese milk. Aeration of the milk at 20° C. acted as a "trigger" for phage action. The phage was specific to one strain of streptococci. Quantitative differences in a minor constituent or qualitative differences in a major constituent of milk may also affect the activity of the phage. There was some evidence of the appearance of what are apparently different phages on one strain of bacterium. Attempts to immunize strains of *S. cremoris* against the phage did not prove successful. H.A.B.

Factors in the Reduction of Methylene Blue in Milk. CHRISTOPHER JAMES JACKSON, Dept. of Bioch., Univ. of Alberta, Edmonton, Canada. J. Dairy Research *7*, 1, p. 31, Jan., 1936.

It was found that milk drawn anaerobically from the udder of the cow by a specially devised apparatus reduces methylene blue almost instantaneously, whereas the same milk exposed to the air will usually take more than 10 hours for reduction.

The oxidation-reduction potential of milk drawn anaerobically into an air-free electrode vessel, was found also to be lower than the same milk exposed to oxygen.

Milk under anaerobic conditions has only a small oxidation-reduction capacity, indicating that a redox system, present in low concentration, is responsible for the reduction of methylene blue. For that reason, it is suggested that the major constituents of milk, protein, fat and sugar, play no

part in the reduction mechanism, but results indicate that lactoflavin may furnish the redox system.

The addition of small amounts of cysteine or glutathione to milk leads to the reduction of methylene-blue, but these substances were not found present in normal milk.

Light in the visible spectrum catalyzes the reduction of methylene blue in milk.

Bacteria may play but an insignificant part in the reduction of methylene blue in milk, though their de-oxygenating effect may be of influence in the commercial application of the test. H.A.B.

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